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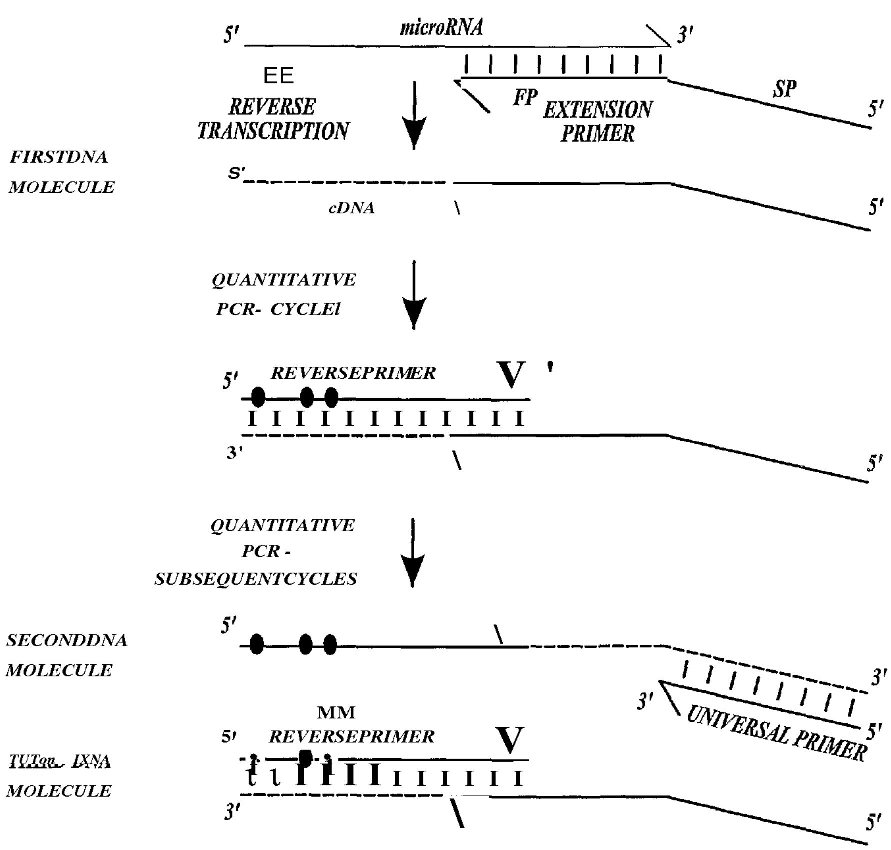
(54) Title: METHODS FOR QUANTITATING SMALL RNA MOLECULES



(57) Abstract: In one aspect, the present invention provides methods for amplifying a microRNA molecule to produce DNA molecules. The methods each include the steps of: (a) using primer extension to make a DNA molecule that is complementary to a target microRNA molecule; and (b) using a universal forward primer and a reverse primer to amplify the DNA molecule to produce amplified DNA molecules. In some embodiments of the method, at least one of the forward primer and the reverse primer comprise at least one-locked nucleic acid molecule.

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METHODS FOR QUANTITATING SMALL RNA MOLECULES

FIELD OF THE INVENTION

The present invention relates to methods of amplifying and quantitating small RNA molecules.

BACKGROUND OF THE INVENTION

RNA interference (RNAi) is an evolutionarily conserved process that functions to inhibit gene expression (Bernstein et al. (2001), *Nature 409:363-6;* Dykxhoorn et al. (2003) *Nat. Rev. MoL Cell. Biol. 4:451-61).* The phenomenon of RNAi was first described in *Caenorhabditis elegans,* where injection of double-stranded RNA (dsRNA) led to efficient sequence-specific gene silencing of the mRNA that was complementary to the dsRNA (Fire et al. (1998) *Nature* 397:806-1 1). RNAi has also been described in plants as a phenomenon called post-transcriptional gene silencing (PTGS)$_3$ which is likely used as a viral defense mechanism (Jorgensen (1990) *Trends Biotechnol.* 5:340-4; Brigneti et al. (1998) *EMBO J.* 77:6739-46; Hamilton & Baulcombe (1999) *Science* 286:950-2).

An early indication that the molecules that regulate PTGS were short RNAs processed from longer dsRNA was the identification of short 2 1 to 22 nucleotide dsRNA derived from the longer dsRNA in plants (Hamilton & Baulcombe (1999) *Science 286:950-2).* This observation was repeated in *Drosophila* embryo extracts where long dsRNA was found processed into 21-25 nucleotide short RNA by the RNase III type enzyme, Dicer (Elbashir et al. (2001) *Nature* 47.7:494-8; Elbashir et al. (2001) TiMSO *J* 20:6877-88; Elbashir et al. (2001) *Genes Dev.* 75:188-200). These observations led Elbashir et al. to test if synthetic 21-25 nucleotide synthetic dsRNAs function to specifically inhibit gene expression in *Drosophila* embryo lysates and mammalian cell

culture (Elbashir et al. (2001) *Nature 411:494-8;* Elbashir et al. (2001) *EMBO J.* 20:6877-88; Elbashir et al. (2001) *Genes Dev.* 75:188-200). They demonstrated that small interfering RNAs (siRNAs) had the ability to specifically inhibit gene expression in mammalian cell culture without induction of the interferon response.

These observations led to the development of techniques for the reduction, or elimination, of expression of specific genes in mammalian cell culture, such as plasmid-based systems that generate hairpin siRNAs (Brummelkamp et al. (2002) *Science 296:550-3;* Paddison et al. (2002) *Genes Dev.* 75:948-58; Paddison et al. (2002) *Proc. Natl. Acad. ScL U.S.A. 99:* 1443-8; Paul et al. 2002) *Nat. Biotechnol.* 20:404-8). siRNA molecules can also be introduced into cells, *in vivo,* to inhibit the expression of specific proteins (see, e.g., Soutschek, J., et al, *Nature 432* (7014):173-178 (2004)).

siRNA molecules have promise both as therapeutic agents for inhibiting the expression of specific proteins, and as targets for drugs that affect the activity of siRNA molecules that function to regulate the expression of proteins involved in a disease state. A first step in developing such therapeutic agents is to measure the amounts of specific siRNA molecules in different cell types within an organism, and thereby construct an "atlas" of siRNA expression within the body. Additionally, it will be useful to measure changes in the amount of specific siRNA molecules in specific cell types in response to a defined stimulus, or in a disease state.

Short RNA molecules are difficult to quantitate. For example, with respect to the use of PCR to amplify and measure the small RNA molecules, most PCR primers are longer than the small RNA molecules, and so it is difficult to design a primer that has significant overlap with a small RNA molecule, and that selectively hybridizes to the small RNA molecule at the temperatures used for primer extension and PCR amplification reactions.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides methods for amplifying a microRNA molecule to produce cDNA molecules. The methods include the steps of: (a) producing a first DNA molecule that is complementary to a target microRNA molecule using primer extension; and (b) amplifying the first DNA molecule to produce amplified DNA molecules using a universal forward primer and a reverse primer. In some embodiments of the method, at least one of the forward primer and the reverse primer comprise at least one locked nucleic acid molecule. It will be understood that, in the practice of the present

invention, typically numerous (e.g., millions) of individual microRNA molecules are amplified in a sample (e.g., a solution of RNA molecules isolated from living cells).

In another aspect, the present invention provides methods for measuring the amount of a target microRNA in a a sample from a living organism. The methods of this aspect of the invention include the step of measuring the amount of a target microRNA molecule in a multiplicity of different cell types within a living organism, wherein the amount of the target microRNA molecule is measured by a method including the steps of: (1) producing a first DNA molecule complementary to the target microRNA molecule in the sample using primer extension; (2) amplifying the first DNA molecule to produce amplified DNA molecules using a universal forward primer and a reverse primer; and (3) measuring the amount of the amplified DNA molecules. In some embodiments of the method, at least one of the forward primer and the reverse primer comprise at least one locked nucleic acid molecule.

In another aspect, the invention provides nucleic acid primer molecules consisting of sequence SEQ ID NO:1 to SEQ ID NO: 499, as shown in TABLE 1, TABLE 2, TABLE 6 and TABLE 7. The primer molecules of the invention can be used as primers for detecting mammalian microRNA target molecules, using the methods of the invention described herein.

In another aspect, the present invention provides kits for detecting at least one mammalian target microRNA, the kits comprising one or more primer sets specific for the detection of a target microRNA, each primer set comprising (1) an extension primer for producing a cDNA molecule complementary to a target microRNA, (2) a universal forward PCR primer for amplifying the cDNA molecule and (3) a reverse PCR primer for amplifying the cDNA molecule. The extension primer comprises a first portion that hybridizes to the target microRNA molecule and a second portion that includes a hybridization sequence for a universal forward PCR primer. The reverse PCR primer comprises a sequence selected to hybridize to a portion of the cDNA molecule. In some embodiments of the kit, at least one of the universal forward and reverse primers include at least one locked nucleic acid molecule. The kits of the invention may be used to practice various embodiments of the methods of the invention.

The present invention is useful, for example, for quantitating specific microRNA molecules within different types of cells in a living organism, or, for example, for

measuring changes in the amount of specific microRNAs in living cells in response to a stimulus (e.g., in response to administration of a drug).

BRIEF DESCRPTION OF THE DRAWINGS

The foregoing aspects and many of the attendant advantages of this invention will become more readily appreciated as the same become better understood by reference to the following detailed description, when taken in conjunction with the accompanying drawings, wherein:

FIGURE 1 shows a flow chart of a representative method of the present invention;

FIGURE 2 graphically illustrates the standard curves for assays specific for the detection of microRNA targets miR-95 and miR-424 as described in EXAMPLE 3;

FIGURE 3A is a histogram plot showing the expression profile of miR-1 across a panel of total RNA isolated from twelve tissues as described in EXAMPLE 5;

FIGURE 3B is a histogram plot showing the expression profile of miR-124 across a panel of total RNA isolated from twelve tissues as described in EXAMPLE 5; and

FIGURE 3C is a histogram plot showing the expression profile of miR-150 across a panel of total RNA isolated from twelve tissues as described in EXAMPLE 5.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

In accordance with the foregoing, in one aspect, the present invention provides methods for amplifying a microRNA molecule to produce cDNA molecules. The methods include the steps of: (a) using primer extension to make a DNA molecule that is complementary to a target microRNA molecule; and (b) using a universal forward primer and a reverse primer to amplify the DNA molecule to produce amplified DNA molecules, hi some embodiments of the method, at least one of the universal forward primer and the reverse primer comprises at least one locked nucleic acid molecule.

As used herein, the term "locked nucleic acid molecule" (abbreviated as LNA molecule) refers to a nucleic acid molecule that includes a 2'-O,4'-C-methylene-β-D-ribofuranosyl moiety. Exemplary 2'-O,4'-C-methylene-β-D-ribofuranosyl moieties, and exemplary LNAs including such moieties, are described, for example, in Petersen, M. and Wengel, J., *Trends in Biotechnology* 2i(2):74-81 (2003) which publication is incorporated herein by reference in its entirety.

As used herein, the term "microRNA" refers to an RNA molecule that has a length in the range of from 21 nucleotides to 25 nucleotides. Some microRNA molecules (e.g., siRNA molecules) function in living cells to regulate gene expression.

Representative method of the invention. FIGURE 1 shows a flowchart of a representative method of the present invention. In the method represented in FIGURE 1, a microRNA is the template for synthesis of a complementary first DNA molecule. The synthesis of the first DNA molecule is primed by an extension primer, and so the first DNA molecule includes the extension primer and newly synthesized DNA (represented by a dotted line in FIGURE 1). The synthesis of DNA is catalyzed by reverse transcriptase.

The extension primer includes a first portion (abbreviated as FP in FIGURE 1) and a second portion (abbreviated as SP in FIGURE 1). The first portion hybridizes to the microRNA target template, and the second portion includes a nucleic acid sequence that hybridizes with a universal forward primer, as described *infra*.

A quantitative polymerase chain reaction is used to make a second DNA molecule that is complementary to the first DNA molecule. The synthesis of the second DNA molecule is primed by the reverse primer that has a sequence that is selected to specifically hybridize to a portion of the target first DNA molecule. Thus, the reverse primer does not hybridize to nucleic acid molecules other than the first DNA molecule. The reverse primer may optionally include at least one LNA molecule located within the portion of the reverse primer that does not overlap with the extension primer. In FIGURE 1, the LNA molecules are represented by shaded ovals.

A universal forward primer hybridizes to the 3' end of the second DNA molecule and primes synthesis of a third DNA molecule. It will be understood that, although a single microRNA molecule, single first DNA molecule, single second DNA molecule, single third DNA molecule and single extension, forward and reverse primers are shown in FIGURE 1, typically the practice of the present invention uses reaction mixtures that include numerous copies (e.g., millions of copies) of each of the foregoing nucleic acid molecules.

The steps of the methods of the present invention are now considered in more detail.

Preparation of microRNA molecules useful as templates. microRNA molecules useful as templates in the methods of the invention can be isolated from any organism (e.g., eukaryote, such as a mammal) or part thereof, including organs, tissues, and/or individual cells (including cultured cells). Any suitable RNA preparation that includes microRNAs can be used, such as total cellular RNA.

RNA may be isolated from cells by procedures that involve lysis of the cells and denaturation of the proteins contained therein. Cells of interest include wild-type cells, drug-exposed wild-type cells, modified cells, and drug-exposed modified cells.

Additional steps may be employed to remove some or all of the DNA. Cell lysis may be accomplished with a nonionic detergent, followed by microcentrifugation to remove the nuclei and hence the bulk of the cellular DNA. In one embodiment, RNA is extracted from cells of the various types of interest using guanidinium thiocyanate lysis followed by CsCl centrifugation to separate the RNA from DNA (see, Chirgwin et al., 1979, *Biochemistry 18:5294-5299).* Separation of RNA from DNA can also be accomplished by organic extraction, for example, with hot phenol or phenol/chloroform/isoamyl alcohol.

If desired, RNase inhibitors may be added to the lysis buffer. Likewise, for certain cell types, it may be desirable to add a protein denaturation/digestion step to the protocol.

The sample of RNA can comprise a multiplicity of different microRNA molecules, each different microRNA molecule having a different nucleotide sequence. In a specific embodiment, the microRNA molecules in the RNA sample comprise at least 100 different nucleotide sequences. In other embodiments, the microRNA molecules of the RNA sample comprise at least 500, 1,000, 5,000, 10,000, 20,000, 30,000, 40,000, 50,000, 60,000, 70,000, 80,000 90,000, or 100,000 different nucleotide sequences.

The methods of the invention may be used to detect the presence of any microRNA. For example, the methods of the invention can be used to detect one or more of the microRNA targets described in a database such as "the miRBase sequence database" as described in Griffith-Jones et al. (2004), *Nucleic Acids Research* 32:D109-D1 11, and Griffith-Jones et al. (2006), *Nucleic Acids Research* 34: D140-D144, which is publically accessible on the World Wide Web at the Wellcome Trust Sanger Institute website at http://microrna.sanger.ac.uk/sequences/. A list of exemplary microRNA targets is also described in the following references: Lagos-Quintana et al., *Curr. Biol.* 12(9):735-9 (2002).

<u>Synthesis of DNA molecules using microRNA molecules as templates</u>. In the practice of the methods of the invention, first DNA molecules are synthesized that are complementary to the microRNA target molecules, and that are composed of an extension primer and newly synthesized DNA (wherein the extension primer primes the

synthesis of the newly synthesized DNA). Individual first DNA molecules can be complementary to a whole microRNA target molecule, or to a portion thereof; although typically an individual first DNA molecule is complementary to a whole microRNA target molecule. Thus, in the practice of the methods of the invention, a population of first DNA molecules is synthesized that includes individual DNA molecules that are each complementary to all, or to a portion, of a target microRNA molecule.

The synthesis of the first DNA molecules is catalyzed by reverse transcriptase. Any reverse transcriptase molecule can be used to synthesize the first DNA molecules, such as those derived from Moloney murine leukemia virus (MMLV-RT), avian myeloblastosis virus (AMV-RT), bovine leukemia virus (BLV-RT), Rous sarcoma virus (RSV) and human immunodeficiency virus (HIV-RT). A reverse transcriptase lacking RNaseH activity (e.g., SUPERSCRIPT III™ sold by Invitrogen, 1600 Faraday Avenue, PO Box 6482, Carlsbad, California 92008) is preferred in order to minimize the amount of double-stranded cDNA synthesized at this stage. The reverse transcriptase molecule should also preferably be thermostable so that the DNA synthesis reaction can be conducted at as high a temperature as possible, while still permitting hybridization of primer to the microRNA target molecules.

<u>Priming the synthesis of the first DNA molecules</u>. The synthesis of the first DNA molecules is primed using an extension primer. Typically, the length of the extension primer is in the range of from 10 nucleotides to 100 nucleotides, such as 20 to 35 nucleotides. The nucleic acid sequence of the extension primer is incorporated into the sequence of each, synthesized, DNA molecule. The extension primer includes a first portion that hybridizes to a portion of the microRNA molecule. Typically the first portion of the extension primer includes the 3'-end of the extension primer. The first portion of the extension primer typically has a length in the range of from 6 nucleotides to 20 nucleotides, such as from 10 nucleotides to 12 nucleotides. In some embodiments, the first portion of the extension primer has a length in the range of from 3 nucleotides to 25 nucleotides.

The extension primer also includes a second portion that typically has a length of from 18 to 25 nucleotides. For example, the second portion of the extension primer can be 20 nucleotides long. The second portion of the extension primer is located 5' to the first portion of the extension primer. The second portion of the extension primer includes at least a portion of the hybridization site for the universal forward primer. For example,

the second portion of the extension primer can include all of the hybridization site for the universal forward primer, or, for example, can include as little as a single nucleotide of the hybridization site for the universal forward primer (the remaining portion of the hybridization site for the forward primer can, for example, be located in the first portion of the extension primer). An exemplary nucleic acid sequence of a second portion of an extension primer is 5' CATGATCAGCTGGGCCAAGA 3' (SEQ ID NO:1).

Amplification of the DNA molecules. In the practice of the methods of the invention, the fist DNA molecules are enzymatically amplified using the polymerase chain reaction. A universal forward primer and a reverse primer are used to prime the polymerase chain reaction. The reverse primer includes a nucleic acid sequence that is selected to specifically hybridize to a portion of a first DNA molecule.

The reverse primer typically has a length in the range of from 10 nucleotides to 100 nucleotides. In some embodiments, the reverse primer has a length in the range of from 12 nucleotides to 20 nucleotides. The nucleotide sequence of the reverse primer is selected to hybridize to a specific target nucleotide sequence under defined hybridization conditions. The reverse primer and extension primer are both present in the PCR reaction mixture, and so the reverse primer should be sufficiently long so that the melting temperature (Tm) is at least 5CPC, but should not be so long that there is extensive overlap with the extension primer which may cause the formation of "primer dimers." "Primer dimers" are formed when the reverse primer hybridizes to the extension primer, and uses the extension primer as a substrate for DNA synthesis, and the extension primer hybridizes to the reverse primer, and uses the reverse primer as a substrate for DNA synthesis. To avoid the formation of "primer dimers," typically the reverse primer and the extension primer are designed so that they do not overlap with each other by more than 6 nucleotides. If it is not possible to make a reverse primer having a Tm of at least 5CPC, and wherein the reverse primer and the extension primer do not overlap by more than 6 nucleotides, then it is preferable to lengthen the reverse primer (since Tm usually increases with increasing oligonucleotide length) and decrease the length of the extension primer.

The reverse primer primes the synthesis of a second DNA molecule that is complementary to the first DNA molecule. The universal forward primer hybridizes to the portion of the second DNA molecule that is complementary to the second portion of the extension primer which is incorporated into all of the first DNA molecules. The

universal forward primer primes the synthesis of third DNA molecules. The universal forward primer typically has a length in the range of from 16 nucleotides to 100 nucleotides. In some embodiments, the universal forward primer has a length in the range of from 16 nucleotides to 30 nucleotides. The universal forward primer may include at least one locked nucleic acid molecule. In some embodiments, the universal forward primer includes from 1 to 25 locked nucleic acid molecules. The nucleic acid sequence of an exemplary universal forward primer is set forth in SEQ ID NO: 13.

In general, the greater the number of amplification cycles during the polymerase chain reaction, the greater the amount of amplified DNA that is obtained. On the other hand, too many amplification cycles (e.g., more than 35 amplification cycles) may result in spurious and unintended amplification of non-target double-stranded DNA. Thus, in some embodiments, a desirable number of amplification cycles is between one and 45 amplification cycles, such as from one to 25 amplification cycles, or such as from five to 15 amplification cycles, or such as ten amplification cycles.

Use of LNA molecules and selection of primer hybridization conditions: hybridization conditions are selected that promote the specific hybridization of a primer molecule to the complementary sequence on a substrate molecule. With respect to the hybridization of a 12 nucleotide first portion of an extension primer to a microRNA, it has been found that specific hybridization occurs at a temperature of 5CPC. Similarly, it has been found that hybridization of a 20 nucleotide universal forward primer to a complementary DNA molecule, and hybridization of a reverse primer (having a length in the range of from 12-20 nucleotides, such as from 14-16 nucleotides) to a complementary DNA molecule occurs at a temperature of 5CPC. By way of example, it is often desirable to design extension, reverse and universal forward primers that each have a hybridization temperature in the range of from 5CPC to 6CPC.

In some embodiments, LNA molecules can be incorporated into at least one of the extension primer, reverse primer, and universal forward primer to raise the Tm of one, or more, of the foregoing primers to at least 5CPC. Incorporation of an LNA molecule into the portion of the reverse primer that hybridizes to the target first DNA molecule, but not to the extension primer, may be useful because this portion of the reverse primer is typically no more than 10 nucleotides in length. For example, the portion of the reverse primer that hybridizes to the target first DNA molecule, but not to the extension primer, may include at least one locked nucleic- acid molecule (e.g., from 1 to 25 locked nucleic

acid molecules). In some embodiments, two or three locked nucleic acid molecules are included within the first 8 nucleotides from the 5' end of the reverse primer.

The number of LNA residues that must be incorporated into a specific primer to raise the Tm to a desired temperature mainly depends on the length of the primer and the nucleotide composition of the primer. A tool for determining the effect on Tm of one or more LNAs in a primer is available on the Internet Web site of Exiqon, Bygstubben 9, DK-2950 Vedbaek, Denmark.

Although one or more LNAs can be included in any of the primers used in the practice of the present invention, it has been found that the efficiency of synthesis of cDNA is low if an LNA is incorporated into the extension primer. While not wishing to be bound by theory, LNAs may inhibit the activity of reverse transcriptase.

Detecting and measuring the amount of the amplified DNA molecules: the amplified DNA molecules can be detected and quantitated by the presence of detectable marker molecules, such as fluorescent molecules. For example, the amplified DNA molecules can be detected and quantitated by the presence of a dye (e.g., SYBR green) that preferentially or exclusively binds to double stranded DNA during the PCR amplification step of the methods of the present invention. For example, Molecular Probes, Inc. (29851 Willow Creek Road, Eugene, OR 97402) sells quantitative PCR reaction mixtures that include SYBR green dye. By way of further example, another dye (referred to as "BEBO") that can be used to label double stranded DNA produced during real-time PCR is described by Bengtsson, M., et al., *Nucleic Acids Research* 37(8):e45 (April 15, 2003), which publication is incorporated herein by reference. Again by way of example, a forward and/or reverse primer that includes a fluorophore and quencher can be used to prime the PCR amplification step of the methods of the present invention. The physical separation of the fluorophore and quencher that occurs after extension of the labeled primer during PCR permits the fluorophore to fluoresce, and the fluorescence can be used to measure the amount of the PCR amplification products. Examples of commercially available primers that include a fluorophore and quencher include Scorpion primers and Uniprimers, which are both sold by Molecular Probes, Inc.

Representative uses of the present invention: The present invention is useful for producing cDNA molecules from microRNA target molecules. The amount of the DNA molecules can be measured which provides a measurement of the amount of target microRNA molecules in the starting material. For example, the methods of the present

invention can be used to measure the amount of specific microRNA molecules (e.g., specific siRNA molecules) in living cells. Again by way of example, the present invention can be used to measure the amount of specific microRNA molecules (e.g., specific siRNA molecules) in different cell types in a living body, thereby producing an "atlas" of the distribution of specific microRNA molecules within the body. Again by way of example, the present invention can be used to measure changes in the amount of specific microRNA molecules (e.g., specific siRNA molecules) in response to a stimulus, such as in response to treatment of a population of living cells with a drug.

Thus, in another aspect, the present invention provides methods for measuring the amount of a target microRNA in a multiplicity of different cell types within a living organism (e.g., to make a microRNA "atlas" of the organism). The methods of this aspect of the invention each include the step of measuring the amount of a target microRNA molecule in a multiplicity of different cell types within a living organism, wherein the amount of the target microRNA molecule is measured by a method comprising the steps of: (1) using primer extension to make a DNA molecule complementary to the target microRNA molecule isolated from a cell type of a living organism; (2) using a universal forward primer and a reverse primer to amplify the DNA molecule to produce amplified DNA molecules, and (3) measuring the amount of the amplified DNA molecules. In some embodiments of the methods, at least one of the forward primer and the reverse primer comprises at least one locked nucleic acid molecule. The measured amounts of amplified DNA molecules can, for example, be stored in an interrogatable database in electronic form, such as on a computer-readable medium (e.g., a floppy disc).

In another aspect, the invention provides nucleic acid primer molecules consisting of sequence SEQ ID NO:1 to SEQ ID NO: 499, as shown in TABLE 1, TABLE 2, TABLE 6 and TABLE 7. The primer molecules of the invention can be used as primers for detecting mammalian microRNA target molecules, using the methods of the invention described herein.

In another aspect, the present invention provides kits for detecting at least one mammalian target microRNA, the kits comprising one or more primer sets specific for the detection of a target microRNA, each primer set comprising (1) an extension primer for producing a cDNA molecule complementary to a target microRNA, (2) a universal forward PCR primer and (3) a reverse PCR primer for amplifying the cDNA molecule. The extension primer comprises a first portion that hybridizes to the target microRNA

molecule and a second portion that includes a hybridization sequence for a universal forward PCR primer. The reverse PCR primer comprises a sequence selected to hybridize to a portion of the cDNA molecule. In some embodiments of the kits, at least one of the universal forward and reverse primers includes at least one locked nucleic acid molecule.

The extension primer, universal forward and reverse primers for inclusion in the kit may be designed to detect any mammalian target microRNA in accordance with the methods described herein. Nonlimiting examples of human target microRNA target molecules and exemplary target-specific extension primers and reverse primers are listed below in TABLE I5 TABLE 2 and TABLE 6. Nonlimiting examples of murine target microRNA target molecules and exemplary target-specific extension primers and reverse primers are listed below in TABLE 7. A nonlimiting example of a universal forward primer is set forth as SEQ ID NO: 13.

In certain embodiments, the ldt includes a set of primers comprising an extension primer, reverse and universal forward primers for a selected target microRNA molecule that each have a hybridization temperature in the range of from 5CPC to 6CPC.

In certain embodiments, the kit includes a plurality of primer sets that may be used to detect a plurality of mammalian microRNA targets, such as two microRNA targets up to several hundred microRNA targets.

In certain embodiments, the kit comprises one or more primer sets capable of detecting at least one or more of the following human microRNA target templates: of miR-1, miR-7, miR-9*, miR-lOa, miR-lOb, miR-15a, miR-15b, miR-16, miR-17-3p, miR-17-5 ρ, miR-1 8, miR-1 9a, miR-1 9b, miR-20, miR-21, miR-22, miR-23a, miR-23b, miR-24, miR-25, miR-26a, miR-26b, miR-27a, miR-28, miR-29a, miR-29b, miR-29c, miR-30a-5p, miR-30b, miR-30c, miR-30d, miR-30e-5p, miR-30e-3p, mJR-31, miR-32, miR-33, miR-34a, miR-34b, miR-34c, miR-92, miR-93, miR-95, miR-96, miR-98, miR-99a, miR-99b, miR-1 00, miR-101, miR-1 03, miR-1 05, miR-106a, miR-107, miR-122, miR-122a, miR-124, miR-124, miR-124a, miR-125a, miR-125b, miR-126, miR-126*, miR-127, miR-128a, miR-128b, miR-129, miR-1 30a, miR-130b, miR-132, miR-133a, miR-133b, miR-134, miR-135a, miR-135b, miR-1 36, miR-1 37, miR-1 38, miR-139, miR-140, miR-141, miR-142-3p, miR-143 5miR-144, miR-145, miR-146, miR-147, miR-148a, miR-148b, miR-149, miR-150, miR-151, miR-152, miR-1 53, miR-154*, miR-154, miR-155, miR-1 8Ia, miR-1 81b, miR-1 8Ic, miR-1 82*, miR-1 82, miR-183, miR-184, miR-

185, miR-186, miR-187, miR-188, miR-189, miR-190, miR-191, miR-192, miR-193, miR-194, miR-195, miR-196a, miR-196b, miR-197, miR-198, miR-199a*, miR-199a, miR-199b, miR-200a, miR-200b, miR-200c, miR-202, miR-203, miR-204, miR-205, miR-206, miR-208, miR-210, miR-211, miR-212, miR-213, miR-213, miR-214, miR-215, miR-216, miR-217, miR-218, miR-220, miR-221, miR-222, miR-223, miR-224, miR-296, miR-299, miR-301, miR-302a*, miR-302a, miR-302b*, miR-302b, miR-302d, miR-302c*, miR-302c, miR-320, miR-323, miR-324-3p, miR-324-5p, miR-325, miR-326, miR-328, miR-330, miR-331, miR-337, miR-338, miR-339, miR-340, miR-342, miR-345, miR-346, miR-363, miR-367, miR-368, miR-370, miR-371, miR-372, miR-373*, miR-373, miR-374, miR-375, miR-376b, miR-378, miR-379, miR-380-5p, miR-380-3p, miR-381, miR-382, miR-383, miR-410, miR-412, miR-422a, miR-422b, miR-423, miR-424, miR-425, miR-429, miR-431, miR-448, miR-449, miR-450, miR-451, Iet7a, Iet7b, Iet7c, Iet7d, Iet7e, Iet7f, Iet7g, Iet7i, miR-376a, and miR-377. The sequences of the above-mentioned microRNA targets are provided in "the miRBase sequence database" as described in Griffith-Jones et al. (2004), *Nucleic Acids Research* 52:D109-DlIl, and Griffith-Jones et al. (2006), *Nucleic Acids Research* 34: D140-D144, which is publically accessible on the World Wide Web at the Wellcome Trust Sanger Institute website at http://microrna.sanger.ac.uk/sequences/.

Exemplary primers for use in accordance with this embodiment of the kit are provided in TABLE 1, TABLE 2 and TABLE 6 below.

In another embodiment, the kit comprises one or more primer sets capable of detecting at least one or more of the following human microRNA target templates: miR-1, miR-7, miR-lOb, miR-26a, miR-26b, miR-29a, miR-30e-3p, miR-95, miR-107, miR-141, miR-143, miR-154*, miR-154, miR-155, miR-181a, miR-181b, miR-181c, miR-190, miR-193, miR-194, miR-195, miR-202, miR-206, miR-208, miR-212, miR-221, miR-222, miR-224, miR-296, miR-299, miR-302c*, miR-302c, miR-320, miR-339, miR363, miR-376b, miR379, miR410, miR412, miR424, miR429, miR431, miR449, miR451, Iet7a, Iet7b, Iet7c, Iet7d, Iet7e, Iet7f, Iet7g, and Iet7i. Exemplary primers for use in accordance with this embodiment of the kit are provided in TABLE 1, TABLE 2 and TABLE 6 below.

In another embodiment, the kit comprises at least one oligonucleotide primer selected from the group consisting of SEQ ID NO: 2 to SEQ ID NO: 493, as shown in TABLE 1, TABLE 2, TABLE 6 and TABLE 7.

In another embodiment, the kit comprises at least one oligonucleotide primer selected from the group consisting of SEQ ID NO: 47, 48, 49, 50, 55, 56, 81, 82, 83, 84, 91, 92, 103, 104, 123, 124, 145, 146, 193, 194, 197, 198, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 239, 240, 247, 248, 253, 254, 255, 256, 257, 258, 277, 278, 285, 286, 287, 288, 293, 294, 301, 302, 309, 310, 311, 312, 315, 316, 317, 318, 319, 320, 333, 334, 335, 336, 337, 338, 359, 360, 369, 370, 389, 390, 393, 394, 405, 406, 407, 408, 415, 416, 419, 420, 421, 422, 425, 426, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 461 and 462, as shown in TABLE 6.

A kit of the invention can also provide reagents for primer extension and amplification reactions. For example, in some embodiments, the kit may further include one or more of the following components: a reverse transcriptase enzyme, a DNA polymerase enzyme, a Tris buffer, a potassium salt (e.g., potassium chloride), a magnesium salt (e.g., magnesium chloride), a reducing agent (e.g., dithiothreitol), and deoxynucleoside triphosphates (dNTPs).

In various embodiments, the kit may include a detection reagent such as SYBR green dye or BEBO dye that preferentially or exclusively binds to double stranded DNA during a PCR amplification step. In other embodiments, the kit may include a forward and/or reverse primer that includes a fluorophore and quencher to measure the amount of the PCR amplification products.

The kit optionally includes instructions for using the kit in the detection and quantitation of one or more mammalian microRNA targets. The kit can also be optionally provided in a suitable housing that is preferably useful for robotic handling in a high throughput manner.

The following examples merely illustrate the best mode now contemplated for practicing the invention, but should not be construed to limit the invention.

EXAMPLE 1

This Example describes a representative method of the invention for producing DNA molecules from microRNA target molecules.

Primer extension was conducted as follows (using InVitrogen Superscript III® reverse transcriptase and following the guidelines that were provided with the enzyme). The following reaction mixture was prepared on ice:

1 µl of 10 mM dNTPs

1 µl of 2 µM extension primer

1- 5 µl of target template

4 µl of "5X cDNA buffer"

1 µl of 0.1 M DTT

1 µl of RNAse OUT

1 µl of Superscript III® enzyme

water to 20 µl

The mixture was incubated at 5CPC for 30 minutes, then 85^{0}C for 5 minutes, then cooled to room temperature and diluted 10-fold with TE (10 mM Tris, pH 7.6, 0.1 mM EDTA).

Real-time PCR was conducted using an ABI 7900 HTS detection system (Applied Biosystems, Foster City, California, U.S.A.) by monitoring SYBR® green fluorescence of double-stranded PCR amplicons as a function of PCR cycle number. A typical 10 µl PCR reaction mixture contained:

5 µl of 2X SYBR® green master mix (ABI)

0.8 µl of 10 µM universal forward primer

0.8 µl of 10 µM reverse primer

1.4 µl of water

2.0 µl of target template (10-fold diluted RT reaction).

The reaction was monitored through 40 cycles of standard "two cycle" PCR (95^{0}C - 15 sec, 60^{0}C - 60 sec) and the fluorescence of the PCR products was measured.

The foregoing method was successfully used in eleven primer extension PCR assays for quantitation of endogenous microRNAs present in a sample of total RNA. The DNA sequences of the extension primers, the universal forward primer sequence, and the LNA substituted reverse primers, used in these 11 assays are shown in TABLE 1.

TABLE 1

Target microRNA	Primer number	Primer Name	DNA sequence (5' to 3')	SEQ ID NO
gene-specific extension primers[1]				
humanb let7a	357	let7aP4	*CATGATCAGCTGGGCCAAGA*AACTATACAACCT	2
human miR-1	337	miR1P5	*CATGATCAGCTGGGCCAAGA*TACATACTTCT	3
human miR-15a	344	miR15aP3	*CATGATCAGCTGGGCCAAGA*CACAAACCATTATG	4
human miR-16	351	miR16P2	*CATGATCAGCTGGGCCAAGA*CGCCAATATTTACGT	5

Target microRNA	Primer number	Primer Name	DNA sequence (5' to 3')	SEQ ID NO
human miR-21	342	miR21P6	*CATGATCAGCTGGGCCAAGA*TCAACATCAGT	6
human miR-24	350	miR24P5	*CATGATCAGCTGGGCCAAGA*CTGTTCCTGCTG	7
human miR-122	222	122-E5F	*CATGATCAGCTGGGCCAAGA*ACAAACACCATTGTCA	8
human miR-124	226	124-E5F	*CATGATCAGCTGGGCCAAGA*TGGCATTCACCGCGTG	9
human miR-143	362	miR143P5	*CATGATCAGCTGGGCCAAGA*TGAGCTACAGTG	10
human miR-145	305	miR145P2	*CATGATCAGCTGGGCCAAGA*AAGGGATTCCTGGGAA	11
human miR-155	367	miR155P3	*CATGATCAGCTGGGCCAAGA*CCCCTATCACGAT	12
[1] - Universal forward primer binding sites are shown in italics. The overlap with the RNA-specific reverse primers are underlined.				
universal forward primer				
	230	E5F	CATGATCAGCTGGGCCAAGA	13
RNA species-specific reverse primers[2]				
human let7a	290	miRlet7a-1,2,3R	TG+AGGT+AGTAGGTTG	14
human miR-1	285	miR1-1,2R	TG+GAA+TG+TAAAGAAGTA	15
human miR-15a	287	miR15aR	TAG+CAG+CACATAATG	16
human miR-16	289	miR16-1,2R	T+AGC+AGCACGTAAA	17
human miR-21	286	miR21R	T+AG+CT+TATCAGACTGAT	18
human miR-24	288	miR24-1,2R	TGG+CTCAGTTCAGC	19
human miR-122	234	122LNAR	T+G+GAG+TGTGACAA	20
human miR-124	235	124LNAR	T+TAA+GGCACGCG	21
human miR-143	291	miR143R	TG+AGA+TGAAGCACTG	22
human miR-145	314	miR145R2	GT+CCAGTTTTCCCA	23
human miR-155	293	miR155R	T+TAA+TG+CTAATCGTGA	24
[2] - LNA molecules are preceded by a "+". Region of overlap of the reverse primers with the corresponding extension primers are underlined.				

The assay was capable of detecting microRNA in a concentration range of from 2 nM to 20 fM. The assays were linear at least up to a concentration of 2 nM of synthetic microRNA (>1,000,000 copies/cell).

EXAMPLE 2

This Example describes the evaluation of the minimum sequence requirements for efficient primer-extension mediated cDNA synthesis using a series of extension primers for microRNA assays having gene specific regions that range in length from 12 to 3 base pairs.

Primer Extension Reactions: Primer extension was conducted using the target molecules miR-195 and miR-215 as follows. The target templates miR-195 and miR-215 were diluted to InM RNA (100,000 copies/cell) in TE zero plus 1OOng/µl total yeast RNA. A no template control (NTC) was prepared with TE zero plus lOOng/µl total yeast RNA.

The reverse transcriptase reactions were carried out as follows (using InVitrogen Superscript III® reverse transcriptase and following the guidelines that were provided with the enzyme) using a series of extension primers for miR-195 (SEQ ID NO: 25-34) and a series of extension primers for miR-215 (SEQ ID NO: 35-44) the sequences of which are shown below in TABLE 2.

The following reaction mixtures were prepared on ice:

Set 1: No Template Control

37.5li water

12.5 µl of 1OmM dNTPs

12.5 µl O.1 mM DTT

50µl of "5X cDNA buffer"

12.5liRNAse OUT

12.5 µl Superscript III® reverse transcriptase enzyme

12.5 µl JjgjtHela cell total RNA (Ambion)

plus 50liof 2µM extension primer

plus 50µl TEzero + yeast RNA

Set 2: Spike-in Template

37.5li water

12.5li of 1OmM dNTPs

12.5 µl O.1 mM DTT

50li of "5X cDNA buffer"

12.5 µl RNAse OUT

12.5µl Superscript III® reverse transcriptase enzyme (InVitrogen)

12.5 µl 1jgµlHela cell total RNA (Ambion)

plus 50µlof 2µM extension primer

plus 50µl 1 nM RNA target template (miR-195 or miR-215) serially diluted in 10-fold increments

The reactions were incubated at 5(JC for 30 minutes, then 85C for 5 minutes, and cooled to 4€ and diluted 10-fold with TE (1OmM Tris, pH 7.6, 0.1 mM EDTA).

Quantitative Real-Time PCR reactions: Following reverse transcription, quadruplicate measurements of cDNA were made by quantitative real-time (qPCR) using an ABI 7900 HTS detection system (Applied Biosystems, Foster City, California, U.S.A.) by monitoring SYBR® green fluorescence of double-stranded PCR amplicons as a function of PCR cycle number. The following reaction mixture was prepared:

5µlof 2X SYBR green master mix (ABI)

0.8µl of 1 OµM universal forward primer (SEQ ID NO: 13)

0.8µlof 1 OµMreverse primer (miR-195RP:SEQ ID NO: 45 or miR215RP: SEQ ID NO: 46)

1.4µl of water

2.0µl of target template (10-fold diluted miR-195 or miR-215 RT reaction)

Quantitative real-time PCR was performed for each sample in quadruplicate, using the manufacturer's recommended conditions. The reactions were monitored through 40 cycles of standard "two cycle" PCR (95°C - 15 sec, 6o°C - 60 sec) and the fluorescence of the PCR products were measured and disassociation curves were generated. The DNA sequences of the extension primers, the universal forward primer sequence, and the LNA substituted reverse primers, used in the miR-195 and miR-215 assays are shown below in TABLE 2. The assay results for miR-195 are shown below in TABLE 3 and the assay results for miR-215 are shown below in TABLE 4.

TABLE 2

Target microRNA	Primer number	Primer Name	DNA sequence (5' to 3')	SEQ ID NO:
gene-specific extension primers[1]				
miR-195	646	mir195-GS1	*CATGATCAGCTGGGCCAAGA*GCCAATATTTCT	25
miR-195	647	mir195-GS2	*CATGATCAGCTGGGCCAAGA*GCCAATATTTC	26
miR-195	648	mir195-GS3	*CATGATCAGCTGGGCCAAGA*GCCAATATTT	27
miR-195	649	mir195-GS4	*CATGATCAGCTGGGCCAAGA*GCCAATATT	28
miR-195	650	mir195-GS5	*CATGATCAGCTGGGCCAAGA*GCCAATAT	29
miR-195	651	mir195-GS6	*CATGATCAGCTGGGCCAAGA*GCCAATA	30
miR-195	652	mir195-GS7	*CATGATCAGCTGGGCCAAGA*GCCAAT	31
miR-195	653	mir195-GS8	*CATGATCAGCTGGGCCAAGA*GCCAA	32
miR-195	654	mir195-GS9	*CATGATCAGCTGGGCCAAGA*GCCA	33
miR-195	655	mir195-GS10	*CATGATCAGCTGGGCCAAGA*GCC	34
miR-215	656	mir215-GS1	*CATGATCAGCTGGGCCAAGA*GTCTGTCAATTC	35
miR-215	657	mir215-GS2	*CATGATCAGCTGGGCCAAGA*GTCTGTCAATT	36
miR-215	658	mir215-GS3	*CATGATCAGCTGGGCCAAGA*GTCTGTCAAT	37
miR-215	659	mir215-GS4	*CATGATCAGCTGGGCCAAGA*GTCTGTCAA	38

Target microRNA	Primer number	Primer Name	DNA sequence (5' to 3')	SEQ ID NO:
miR-215	660	mir215-GS5	*CATGATCAGCTGGGCCAAGA*GTCTGTCA	39
miR-215	661	mir215-GS6	*CATGATCAGCTGGGCCAAGA*GTCTGTC	40
miR-215	662	mir215-GS7	*CATGATCAGCTGGGCCAAGA*GTCTGT	41
miR-215	663	mir215-GS8	*CATGATCAGCTGGGCCAAGA*GTCTG	42
miR-215	664	mir215-GS9	*CATGATCAGCTGGGCCAAGA*GTCT	43
miR-215	665	mir215-GS10	*CATGATCAGCTGGGCCAAGA*GTC	44
[1] - Universal forward primer binding sites are shown in italics.				
RNA species-specific reverse primers[2]				
miR-195	442	mir195RP	T+AGC+AGCACAGAAAT	45
miR-215	446	mir215RP	A+T+GA+CCTATGAATTG	46
[2] - The "+" symbol precedes the LNA molecules.				

Results:

The sensitivity of each assay was measured by the cycle threshold (Ct) value which is defined as the cycle count at which fluorescence was detected in an assay containing microRNA target template. The lower this Ct value (e.g. the fewer number of cycles), the more sensitive was the assay. For microRNA samples, it was generally observed that while samples that contain template and no template controls both eventually cross the detection threshold, the samples with template do so at a much lower cycle number. The ΔCt value is the difference between the number of cycles (Ct) between template containing samples and no template controls, and serves as a measure of the dynamic range of the assay. Assays with a high dynamic range allow measurements of very low microRNA copy numbers. Accordingly, desirable

characteristics of a microRNA detection assay include high sensitivity (low Ct value) and broad dynamic range (ΔCt $\geq$ 12) between the signal of a sample containing target template and a no template background control sample.

The results of the miR195 and miR215 assays using extension primers having a gene specific portion ranging in size from 12 nucleotides to 3 nucleotides are shown below in TABLE 3 and TABLE 4, respectively. The results of these experiments unexpectedly demonstrate that gene-specific priming sequences as short as 3 nucleotides exhibit template specific priming. For both the miR-195 assay sets (shown in TABLE 3) and the miR-215 assay sets (shown in TABLE 4), the results demonstrate that the dynamic range (ΔCt) for both sets of assays are fairly consistent for extension primers having gene specific regions that are greater or equal to 8 nucleotides in length. The dynamic range of the assay (ΔCt) begins to decrease for extension primers having gene specific regions below 8 nucleotides, with a reduction in assay specificity below 7 nucleotides in the miR-195 assays, and below 6 nucleotides in the miR-215 assays. A melting point analysis of the miR-215 samples demonstrated that even at 3 nucleotides, there is specific PCR product present in the plus template samples (data not shown). Taken together, these data demonstrate that the gene specific region of extension primers is ideally $\geq$ 8 nucleotides, but can be as short as 3 nucleotides in length.

TABLE 3: miR195 Assay Results

GS Primer Length	Ct: No Template Control	Ct: Plus Template	Δ Ct
12	34.83	20.00	14.82
12	34.19	19.9	14.3
11	40.0	19.8	20.2
10	36.45	21.2	15.2
9	36.40	22.2	14.2
8	40.0	23.73	16.27
7	36.70	25.96	10.73
6	30.95	26.58	4.37

GS Primer Length	Ct: No Template Control	Ct: Plus Template	Δ Ct
5	30.98	31.71	-0.732
4	32.92	33.28	-0.364
3	35.98	35.38	-0.605
Ct=the cycle count where the fluorescence exceeds the threshold of detection. ΔCt = the difference between the Ct value with template and no template.			

TABLE 4: miR215 Assay Results

GS Primer Length	Ct: No Template Control	Ct: Plus Template	Δ Ct
12	33.4	13.57	19.83
12	33.93	14.15	19.77
11	35.51	15.76	19.75
10	35.33	15.49	19.84
9	36.02	16.84	19.18
8	35.79	17.07	18.72
7	32.29	17.58	14.71
6	34.38	20.62	13.75
5	34.41	28.65	5.75
4	36.36	33.92	2.44
3	35.09	33.38	1.70
Ct=the cycle count where the fluorescence exceeds the threshold of detection. ΔCt = the difference between the Ct value with template and no template.			

EXAMPLE 3

This Example describes assays and primer sets designed for quantitative analysis of human microRNA expression patterns.

Primer Design :

microRNA target templates: the sequence of the target templates as described herein are publically available accessible on the World Wide Web at the Wellcome Trust Sanger Institute website in the "miRBase sequence database" as described in Griffith- Jones et al. (2004), *Nucleic Acids Research* 52:D1O9-D111 and and Griffith-Jones et al. (2006), *Nucleic Acids Research* 34: D140-D144.

Extension primers: gene specific primers for primer extension of a microRNA to form a cDNA followed by quantitative PCR (qPCR) amplification were designed to (1) convert the RNA template into cDNA; (2) to introduce a "universal" PCR binding site (SEQ ID NO: 1) to one end of the cDNA molecule; and (3) to extend the length of the cDNA to facilitate subsequent monitoring by qPCR.

Reverse primers: unmodified reverse primers and locked nucleic acid (LNA) containing reverse primers (RP) were designed to quantify the primer-extended, full length cDNA in combination with a generic universal forward primer (SEQ ID NO:13). For the locked nucleic acid containing reverse primers, two or three LNA modified bases were substituted within the first 8 nucleotides from the 5' end of the reverse primer oligonucleotide, as shown below in the exemplary reverse primer sequences provided in TABLE 6. The LNA base substitutions were selected to raise the predicted Tm of the primer by the highest amount, and the final predicted Tm of the selected primers were specified to be preferably less than or equal to *5SO*.

An example describing an assay utilizing an exemplary set of primers the detection of miR-95 and miR-424 is described below.

Primer Extension Reactions : primer extension was conducted using DNA templates corresponding to miR-95 and miR-424 as follows. The DNA templates were diluted to 0 nM, 1 nM, 100 pM, 10 pM and 1 pM dilutions in TE zero (10 mM Tris ρH7.6, 0.1 mM EDTA) plus lOOngjU yeast total RNA (Ambion, Austin TX).

The reverse transcriptase reactions were carried out using the following primers:

Extension primers: (diluted to 500 nM)

miR-95GSP CATGATCAGCTGGGCCAAGATGCTCAATAA (SEQ ID NO: 123)

miR-424GSP CATGATCAGCTGGGCCAAGATTCAAAACAT (SEQ ID NO:415)

Reverse primers: (diluted to 10 mM).

miR-95_RP4 TT+CAAC+GGGTATTTATTGA (SEQ ID NO: 124)

miR-424RP2 C+AG+CAGCAATTCATGTTTT (SEQ ID NO: 416)

Reverse Transcription (per reaction) :

2 µl water

2liof "5X cDNA buffer" (InVitrogen, Carlsbad, CA)

0.5 µl of 0.1 mM DTT (InVitrogen, Carlsbad, CA)

0.5 µl of 10 mM dNTPs (InVitrogen, Carlsbad, CA)

0.5 µl RNAse OUT (InVitrogen, Carlsbad, CA)

0.5 µl Superscript III'® reverse transcriptase enzyme (InVitrogen, Carlsbad, CA)

2 µl of extension primer plus 2 µl of template dilution.

The reactions were mixed and incubated at 5CPC for 30 minutes, then 85C for 5 minutes, and cooled to 4C and diluted 10-fold with TE zero.

Quantitative Real-Time PCR Reactions: (per reaction)

5 µl 2X SYBR mix (Applied Biosystems, Foster City, CA)

1.4 µl water

0.8 µl universal primer (CATGATCAGCTGGGCCAAGA (SEQ ID NO: 13))

2.0 µl of diluted reverse transcription (RT) product from above.

Quantitative real-time PCR was performed for each sample in quadruplicate, using the manufacturer's recommended conditions. The reactions were monitored through 40 cycles of standard "two cycle" PCR (95 ^{0}C - 15 sec, 60 ^{0}C - 60 sec) and the fluorescence of the PCR products were measured and disassociation curves were generated. The DNA sequences of the extension primers, the universal forward primer sequence, and the LNA substituted reverse primers, used in the representative miR-95 and miR-424 assays as well as primer sets for 212 different human microRNA templates are shown below in TABLE 6. Primer sets for assays requiring extensive testing and design modification to achieve a sensitive assay with a high dynamic range are indicated in TABLE 6 with the symbol # following the primer name.

Results:

TABLE 5 shows the Ct values (averaged from four samples) from the miR-95 and miR-424 assays, which are plotted in the graph shown in FIGURE 2. The results of these assays are provided as representative examples in order to explain the significance of the assay parameters shown in TABLE 6 designated as slope (column 6), intercept (column 7) and background (column 8).

As shown in TABLE 5, the Ct value for each template at various concentrations is provided. The Ct values (x-axis) are plotted as a function of template concentration (y-axis) to generate a standard curve for each assay, as shown in FIGURE 2. The slope and intercept define the assay measurement characteristics that permit an estimation of number of copies/cell for each microRNA. For example, when the Ct values for 50 μg total RNA input for the miR-95 assay are plotted, a standard curve is generated with a slope and intercept of -.03569 and 9.655, respectively. When these standard curve parameters are applied to the Ct of an unknown sample (x), they yield log 10 (copies/20pg total RNA) (y). Because the average cell yields 20 pg of total RNA, these measurements equate to copies of microRNA/cell. The background provides an estimate of the minimum copy number that can be measured in a sample and is computed by inserting the no template control (NTC) value into this equation. In this example, as shown in TABLE 6, miR-95 yields a background of 1.68 copies/20 pg at 50 μg of RNA input.

As further shown in TABLE 6, reverse primers that do not contain LNA may also be used in accordance with the methods of the invention. See, e.g. SEQ ID NO: 494-499. The sensitivity and dynamic range of the assays using non-LNA containing reverse primers SEQ ID NO: 494-499, yielded similar results to the corresponding assays using LNA-containing reverse primers.

TABLE 5

Ct Values (averaged from four samples)						
Template concentration	10 nM	I nM	0.1 nM	0.01 nM	0.001 nM	NTC
copies/20 pg RNA (50 μg input)	500,000	50,000	5000	500	50	

Ct Values (averaged from four samples)						
Template concentration	10 nM	1 nM	0.1 nM	0.01 nM	0.001 nM	NTC
copies/20 pg RNA (5 µg input)	5,000,000	500,000	50,000	5000	500	
miR-95	11.71572163	14.17978	17.46353	19.97259	23.33171	27.44383
miR-424	10.47708975	12.76806	15.69251	18.53729	21.56897	23.2813
log10 (copies for 50 µg input)	5.698970004	4.69897	3.69897	2.69897	1.69897	

TABLE 6: Primers to detect human microRNA target templates

Human Target micro RNA	Extension Primer Name	Extension Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Slope	Intercept	Background RNA input 50ug	5ug
# denotes primers for assays that required extensive testing and primer design modification to achieve optimal assay results including high sensitivity and high dynamic range.								
miR-1	miR1GSP10#	CATGATCAGCTGGGCCAAGATACATACTTC SEQ ID NO:47	miR-1RP#	T+G+GAA+TG+TAAAGAAGT SEQ ID NO:48	-0.2758	8.3225	2.44	24.36
miR-7	miR-7GSP10#	CATGATCAGCTGGGCCAAGACAACAAAATC SEQ ID NO:49	miR-7_RP6#	T+GGAA+GACTAGTGATTTT SEQ ID NO:50	-0.2982	10.435	11.70	116.99
miR-9*	miR-9*GSP	CATGATCAGCTGGGCCAAGAACTTTCGGTT SEQ ID NO:51	miR-9*RP	TAAA+GCT+AGATAACCG SEQ ID NO:52	-0.2405	8.9145	3.71	37.15
miR-10a	miR-10aGSP	CATGATCAGCTGGGCCAAGACACAAATTCG SEQ ID NO:53	miR-10aRP	T+AC+CCTGTAGATCCG SEQ ID NO:54	-0.2755	8.6976	0.09	0.94
miR-10b	miR-10b_GSP11#	CATGATCAGCTGGGCCAAGAACAAATTCGGT SEQ ID NO:55	miR-10b_RP2#	TA+CCC+TGT+AGAACCGA SEQ ID NO:56	-0.3505	8.7109	0.55	5.52
miR-15a	miR-15aGSP	CATGATCAGCTGGGCCAAGACACAAACCAT SEQ ID NO:57	miR-15aRP	T+AG+CAGCACATAATG SEQ ID NO:58	-0.2831	8.4519	4.40	44.01
miR-15b	miR-15bGSP2	CATGATCAGCTGGGCCAAGATGTAAACCA SEQ ID NO:59	miR-15bRP	T+AG+CAGCACATCAT SEQ ID NO:60	-0.2903	8.4206	0.18	1.84
miR-16	miR-16GSP2	CATGATCAGCTGGGCCAAGACGCCAATAT SEQ ID NO:61	miR-16RP	T+AG+CAGCACGTAAA SEQ ID NO:62	-0.2542	9.3689	1.64	16.42
miR-17-3p	miR-17-3pGSP	CATGATCAGCTGGGCCAAGAACAAGTGCCT SEQ ID NO:63	miR-17-3pRP	A+CT+GCAGTGAAGGC SEQ ID NO:64	-0.2972	8.2625	1.08	10.78
miR-17-5p	miR-17-5pGSP2	CATGATCAGCTGGGCCAAGAACTACCTGC SEQ ID NO:65	miR-17-5pRP	C+AA+AGTGCTTACAGTG SEQ ID NO:66	-0.2956	7.9101	0.13	1.32

Human Target micro RNA	Extension Primer Name	Extension Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Slope	Intercept	Background RNA input 50ug	5ug
iiR-19a	miR-19aGSP2	CATGATCAGCTGGGCCAAGATCAGTTTTG SEQ ID NO:67	miR-19aRP	TG+TG+CAAATCTATGC SEQ ID NO:68	-0.2984	9.461	0.02	0.23
..iR-19b	miR-19bGSP	CATGATCAGCTGGGCCAAGATCAGTTTTGC SEQ ID NO:69	miR-19bRP	TG+TG+CAAATCCATG SEQ ID NO:70	-0.294	8.1434	2.26	22.55
miR-20	miR-20GSP3	CATGATCAGCTGGGCCAAGACTACCTGC SEQ ID NO:71	miR-20RP	T+AA+AGTGCTTATAGTGCA SEQ ID NO:72	-0.2979	7.9929	0.16	1.60
miR-21	miR-21GSP2	CATGATCAGCTGGGCCAAGATCAACATCA SEQ ID NO: 73	miR-21RP	T+AG+CTTATCAGACTGATG SEQ ID NO:74	-0.2849	8.1624	1.80	17.99
miR-23a	miR-23aGSP	CATGATCAGCTGGGCCAAGAGGAAATCCCT SEQ ID NO:75	miR-23aRP	A+TC+ACATTGCCAGG SEQ ID NO:76	-0.3172	9.4253	2.41	24.08
miR-23b	miR-23bGSP	CATGATCAGCTGGGCCAAGAGGTAATCCCT SEQ ID NO:77	miR-23bRP	A+TC+ACATTGCCAGG SEQ ID NO:78	-0.2944	9.0985	5.39	53.85
miR-25	miR-25GSP	CATGATCAGCTGGGCCAAGATCAGACCGAG SEQ ID NO:79	miR-25RP	C+AT+TGCACTTGTCTC SEQ ID NO:80	-0.3009	8.2482	1.52	15.19
miR-26a	miR-26aGSP9#	CATGATCAGCTGGGCCAAGAGCCTATCCT SEQ ID NO:81	miR-26aRP2#	TT+CA+AGTAATCCAGGAT SEQ ID NO:82	-0.2807	8.558	0.26	2.56
miR-26b	miR-26bGSP9#	CATGATCAGCTGGGCCAAGAAACCTATCC SEQ ID NO:83	miR-26bRP2#	TT+CA+AGT+AATTCAGGAT SEQ ID NO:84	-0.2831	8.7885	0.37	3.67
miR-27a	miR-27aGSP	CATGATCAGCTGGGCCAAGAGCGGAACTTA SEQ ID NO:85	miR-27aRP	TT+CA+CAGTGGCTAA SEQ ID NO:86	-0.2765	9.5239	5.15	51.51
miR-27b	miR-27bGSP	CATGATCAGCTGGGCCAAGAGCAGAACTTA SEQ ID NO:87	miR-27bRP	TT+CA+CAGTGGCTAA SEQ ID NO:88	-0.28	9.5483	5.97	59.71
miR-28	miR-28GSP	CATGATCAGCTGGGCCAAGACTCAATAGAC SEQ ID NO:89	miR-28RP	A+AG+GAGCTCACAGT SEQ ID NO:90	-0.3226	10.071	7.19	71.87

Human Target micro RNA	Extension Primer Name	Extension Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Slope	Intercept	Background RNA input 50ug	5ug
iR-29a	miR-29aGSP8#	CATGATCAGCTGGGCCAAGAAACCGATT SEQ ID NO:91	miR-29aRP2#	T+AG+CACCATCTGAAAT SEQ ID NO:92	-0.29	8.8731	0.04	0.38
iR-29b	miR-29bGSP2	CATGATCAGCTGGGCCAAGAAACACTGAT SEQ ID NO:93	miR-29bRP2	T+AG+CACCATTTGAAATCA G SEQ ID NO:94	-0.3162	9.6276	3.56	35.57
miR-30a-5p	miR-30a-5pGSP	CATGATCAGCTGGGCCAAGACTTCCAGTCG SEQ ID NO:95	miR-30a-5pRP	T+GT+AAACATCCTCGAC SEQ ID NO:96	-0.2772	9.0694	1.92	19.16
miR-30b	miR-30bGSP	CATGATCAGCTGGGCCAAGAAGCTGAGTGT SEQ ID NO:97	miR-30bRP	TGT+AAA+CATCCTACACT SEQ ID NO:98	-0.2621	8.5974	0.11	1.13
miR-30c	miR-30cGSP	CATGATCAGCTGGGCCAAGAGCTGAGAGTG SEQ ID NO:99	miR-30cRP	TGT+AAA+CATCCTACACT SEQ ID NO:100	-0.2703	8.699	0.15	1.48
miR-30d	miR-30dGSP	CATGATCAGCTGGGCCAAGACTTCCAGTCG SEQ ID NO:101	miR-30dRP	T+GTAAA+CATCCCCG SEQ ID NO:102	-0.2506	9.3875	0.23	2.31
miR-30e-3p	miR-30e-3pGSP9#	CATGATCAGCTGGGCCAAGAGCTGTAAAC SEQ ID NO:103	miR-30e-3pRP5#	CTTT+CAGT+CGGATGTTT SEQ ID NO:104	-0.325	11.144	6.37	63.70
miR-30e-5p	miR-30e-5pGSP	CATGATCAGCTGGGCCAAGATCCAGTCAAG SEQ ID NO:105	miR-30e-5pRP	TG+TAAA+CATCCTTGAC SEQ ID NO:106	-0.2732	8.1604	8.50	85.03
miR-31	miR-31GSP	CATGATCAGCTGGGCCAAGACAGCTATGCC SEQ ID NO:107	miR-31RP	G+GC+AAGATGCTGGC SEQ ID NO:108	-0.3068	8.2605	3.74	37.43
miR-32	miR-32GSP	CATGATCAGCTGGGCCAAGAGCAACTTAGT SEQ ID NO:109	miR-32RP	TATTG+CA+CATTACTAAG SEQ ID NO:110	-0.2785	8.9581	0.39	3.93
miR-33	miR-33GSP2	CATGATCAGCTGGGCCAAGACAATGCAAC SEQ ID NO:111	miR-33RP	G+TG+CATTGTAGTTGC SEQ ID NO:112	-0.3031	8.42	2.81	28.14
miR-34a	miR-34aGSP	CATGATCAGCTGGGCCAAGAAACAACCAGC SEQ ID NO:113	miR-34aRP	T+GG+CAGTGTCTTAG SEQ ID NO:114	-0.3062	9.1522	2.40	23.99

Human Target micro RNA	Extension Primer Name	Extension Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Slope	Intercept	Background RNA input 50ug	5ug
iiR-34b	miR-34bGSP	CATGATCAGCTGGGCCAAGACAATCAGCTA SEQ ID NO:115	miR-34bRP	TA+GG+CAGTGTCATT SEQ ID NO:116	-0.3208	9.054	0.04	0.37
iiR-34c	miR-34cGSP	CATGATCAGCTGGGCCAAGAGCAATCAGCT SEQ ID NO:117	miR-34cRP	A+GG+CAGTGTAGTTA SEQ ID NO:118	-0.2995	10.14	1.08	10.83
miR-92	miR-92GSP	CATGATCAGCTGGGCCAAGACAGGCCGGGA SEQ ID NO:119	miR-92RP	T+AT+TGCACTTGTCCC SEQ ID NO:120	-0.3012	8.6908	8.92	89.17
miR-93	miR-93GSP	CATGATCAGCTGGGCCAAGACTACCTGCAC SEQ ID NO:121	miR-93RP	AA+AG+TGCTGTTCGT SEQ ID NO:122	-0.3025	7.9933	4.63	46.30
miR-95	miR-95GSP#	CATGATCAGCTGGGCCAAGATGCTCAATAA SEQ ID NO:123	miR-95_RP4#	TT+CAAC+GGGTATTTATTG A SEQ ID NO:124	-0.3436	9.655	1.68	16.80
miR-96	miR-96GSP	CATGATCAGCTGGGCCAAGAGCAAAAATGT SEQ ID NO:125	miR-96RP	T+TT+GGCACTAGCAC SEQ ID NO:126	-0.2968	9.2611	0.00	0.05
miR-98	miR-98GSP	CATGATCAGCTGGGCCAAGAAACAATACAA SEQ ID NO:127	miR-98RP	TGA+GGT+AGTAAGTTG SEQ ID NO:128	-0.2797	9.5654	1.05	10.48
miR-99a	miR-99aGSP	CATGATCAGCTGGGCCAAGACACAAGATCG SEQ ID NO:129	miR-99aRP	A+AC+CCGTAGATCCG SEQ ID NO:130	-0.2768	8.781	0.21	2.08
miR-99b	miR-99bGSP	CATGATCAGCTGGGCCAAGACGCAAGGTCG SEQ ID NO:131	miR-99bRP	C+AC+CCGTAGAACCG SEQ ID NO:132	-0.2747	7.9855	0.25	2.53
miR-100	miR-100GSP	CATGATCAGCTGGGCCAAGACACAAGTTCG SEQ ID NO:133	miR-100RP	A+AC+CCGTAGATCCG SEQ ID NO:134	-0.2902	8.669	0.04	0.35
miR-101	miR-101GSP	CATGATCAGCTGGGCCAAGACTTCAGTTAT SEQ ID NO:135	miR-101RP	TA+CAG+TACTGTGATAACT SEQ ID NO:136	-0.3023	8.2976	0.46	4.63
miR-103	miR-103GSP	CATGATCAGCTGGGCCAAGATCATAGCCCT SEQ ID NO:137	miR-103RP	A+GC+AGCATTGTACA SEQ ID NO:138	-0.3107	8.5776	0.02	0.21

Human Target micro RNA	Extension Primer Name	Extension Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Slope	Intercept	Background RNA input 50ug	5ug
iR-105	miR-105GSP	CATGATCAGCTGGGCCAAGAACAGGAGTCT SEQ ID NO:139	miR-105RP	T+CAAA+TGCTCAGACT SEQ ID NO:140	-0.2667	8.9832	0.93	9.28
..iR-106a	miR-106aGSP	CATGATCAGCTGGGCCAAGAGCTACCTGCA SEQ ID NO:141	miR-106aRP	AAA+AG+TGCTTACAGTG SEQ ID NO:142	-0.3107	8.358	0.03	0.31
miR-106b	miR-106bGSP	CATGATCAGCTGGGCCAAGAATCTGCACTG SEQ ID NO:143	miR-106bRP	T+AAAG+TGCTGACAGT SEQ ID NO:144	-0.2978	8.7838	0.10	1.04
miR-107	miR-107GSP8#	CATGATCAGCTGGGCCAAGATGATAGCC SEQ ID NO:145	miR-107RP2#	A+GC+AGCATTGTACAG SEQ ID NO:146	-0.304	9.1666	0.34	3.41
miR-122a	miR-122aGSP	CATGATCAGCTGGGCCAAGAACAAACACCA SEQ ID NO:147	miR-122aRP	T+GG+AGTGTGACAAT SEQ ID NO:148	-0.3016	8.1479	0.06	0.58
miR-124a	miR-124aGSP	CATGATCAGCTGGGCCAAGATGGCATTCAC SEQ ID NO:149	miR-124aRP	T+TA+AGGCACGCGGT SEQ ID NO:150	-0.3013	8.6906	0.56	5.63
miR-125a	miR-125aGSP	CATGATCAGCTGGGCCAAGACACAGGTTAA SEQ ID NO:151	miR-125aRP	T+CC+CTGAGACCCTT SEQ ID NO:152	-0.2938	8.6754	0.09	0.91
miR-125b	miR-125bGSP	CATGATCAGCTGGGCCAAGATCACAAGTTA SEQ ID NO:153	miR-125bRP	T+CC+CTGAGACCCTA SEQ ID NO:154	-0.283	8.1251	0.20	1.99
miR-126	miR-126GSP	CATGATCAGCTGGGCCAAGAGCATTATTAC SEQ ID NO:155	miR-126RP	T+CG+TACCGTGAGTA SEQ ID NO:156	-0.26	8.937	0.18	1.80
miR-126*	miR-126*GSP3	CATGATCAGCTGGGCCAAGACGCGTACC SEQ ID NO:157	miR-126*RP	C+ATT+ATTA+CTTTTGGTA CG SEQ ID NO:158	-0.2969	8.184	3.58	35.78
miR-127	miR-127GSP	CATGATCAGCTGGGCCAAGAAGCCAAGCTC SEQ ID NO:159	miR-127RP	T+CG+GATCCGTCTGA SEQ ID NO:160	-0.2432	9.1013	1.11	11.13
miR-128a	miR-128aGSP	CATGATCAGCTGGGCCAAGAAAAAGAGACC SEQ ID NO:161	miR-128aRP	T+CA+CAGTGAACCGG SEQ ID NO:162	-0.2866	8.0867	0.16	1.60

Human Target micro RNA	Extension Primer Name	Extension Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Slope	Intercept	Background RNA input 50ug	5ug
iR-128b	miR-128bGSP	CATGATCAGCTGGGCCAAGAGAAAGAGACC SEQ ID NO:163	miR-128bRP	T+CA+CAGTGAACCGG SEQ ID NO:164	-0.2923	8.0608	0.07	0.74
miR-129	miR-129GSP	CATGATCAGCTGGGCCAAGAGCAAGCCCAG SEQ ID NO:165	miR-129RP	CTTTT+TG+CGGTCTG SEQ ID NO:166	-0.2942	9.7731	0.88	8.85
miR-130a	miR-130aGSP	CATGATCAGCTGGGCCAAGAATGCCCTTTT SEQ ID NO:167	miR-130aRP	C+AG+TGCAATGTTAAAAG SEQ ID NO:168	-0.2943	8.7465	1.28	12.78
miR-130b	miR-130bGSP	CATGATCAGCTGGGCCAAGAATGCCCTTTC SEQ ID NO:169	miR-130bRP	C+AG+TGCAATGATGA SEQ ID NO:170	-0.2377	9.1403	3.14	31.44
miR-132	miR-132GSP	CATGATCAGCTGGGCCAAGACGACCATGGC SEQ ID NO:171	miR-132RP	T+AA+CAGTCTACAGCC SEQ ID NO:172	-0.2948	8.1167	0.11	1.13
miR-133a	miR-133aGSP	CATGATCAGCTGGGCCAAGAACAGCTGGTT SEQ ID NO:173	miR-133aRP	T+TG+GTCCCCTTCAA SEQ ID NO:174	-0.295	9.3679	0.10	1.04
miR-133b	miR-133bGSP	CATGATCAGCTGGGCCAAGATAGCTGGTTG SEQ ID NO:175	miR-133bRP	T+TG+GTCCCCTTCAA SEQ ID NO:176	-0.3062	8.3649	0.02	0.18
miR-134	miR-134GSP	CATGATCAGCTGGGCCAAGACCCTCTGGTC SEQ ID NO:177	miR-134RP	T+GT+GACTGGTTGAC SEQ ID NO:178	-0.2965	9.0483	0.14	1.39
miR-135a	miR-135aGSP	CATGATCAGCTGGGCCAAGATCACATAGGA SEQ ID NO:179	miR-135aRP	T+AT+GGCTTTTTATTCCT SEQ ID NO:180	-0.2914	8.092	1.75	17.50
miR-135b	miR-135bGSP	CATGATCAGCTGGGCCAAGACACATAGGAA SEQ ID NO:181	miR-135bRP	T+AT+GGCTTTTCATTCC SEQ ID NO:182	-0.2962	7.8986	0.05	0.49
miR-136	miR-136GSP	CATGATCAGCTGGGCCAAGATCCATCATCA SEQ ID NO:183	miR-136RP	A+CT+CCATTTGTTTTGATG SEQ ID NO:184	-0.3616	10.229	0.68	6.77
miR-137	miR-137GSP	CATGATCAGCTGGGCCAAGACTACGCGTAT SEQ ID NO:185	miR-137RP	T+AT+TGCTTAAGAATACGC SEQ ID NO:186	-0.2876	8.234	8.57	85.71

Human Target micro RNA	Extension Primer Name	Extension Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Slope	Intercept	Background RNA input 50ug	5ug
iiR-138	miR-138GSP2	CATGATCAGCTGGGCCAAGACGGCCTGAT SEQ ID NO:187	miR-138RP	A+GC+TGGTGTTGTGA SEQ ID NO:188	-0.3023	9.0814	0.22	2.19
iiR-139	miR-139GSP	CATGATCAGCTGGGCCAAGAAGACACGTGC SEQ ID NO:189	miR-139RP	T+CT+ACAGTGCACGT SEQ ID NO:190	-0.2983	8.1141	6.92	69.21
miR-140	miR-140GSP	CATGATCAGCTGGGCCAAGACTACCATAGG SEQ ID NO:191	miR-140RP	A+GT+GGTTTTACCCT SEQ ID NO:192	-0.2312	8.3231	0.13	1.34
miR-141	miR-141GSP9#	CATGATCAGCTGGGCCAAGACCATCTTTA SEQ ID NO:193	miR-141RP2#	TAA+CAC+TGTCTGGTAA SEQ ID NO:194	-0.2805	9.6671	0.13	1.26
miR-142-3p	miR-142-3pGSP3	CATGATCAGCTGGGCCAAGATCCATAAA SEQ ID NO:195	miR-142-3pRP	TGT+AG+TGTTTCCTACT SEQ ID NO:196	-0.2976	8.4046	0.03	0.27
miR-143	miR-143GSP8#	CATGATCAGCTGGGCCAAGATGAGCTAC SEQ ID NO:197	miR-143RP2#	T+GA+GATGAAGCACTG SEQ ID NO:198	-0.3008	9.2675	0.37	3.71
miR-144	miR-144GSP2	CATGATCAGCTGGGCCAAGACTAGTACAT SEQ ID NO:199	miR-144RP	TA+CA+GTAT+AGATGATG SEQ ID NO:200	-0.2407	9.4441	0.95	9.52
miR-145	miR-145GSP2	CATGATCAGCTGGGCCAAGAAAGGGATTC SEQ ID NO:201	miR-145RP	G+TC+CAGTTTTCCCA SEQ ID NO:202	-0.2937	8.0791	0.39	3.86
miR-146	miR-146GSP3	CATGATCAGCTGGGCCAAGAAACCCATG SEQ ID NO:203	miR-146RP	T+GA+GAACTGAATTCCA SEQ ID NO:204	-0.2861	8.8246	0.08	0.75
miR-147	miR-147GSP	CATGATCAGCTGGGCCAAGAGCAGAAGCAT SEQ ID NO:205	miR-147RP	G+TG+TGTGGAAATGC SEQ ID NO:206	-0.2989	8.8866	1.65	16.47
miR-148a	miR-148aGSP2	CATGATCAGCTGGGCCAAGAACAAAGTTC SEQ ID NO:207	miR-148aRP2	T+CA+GTGCACTACAGAACT SEQ ID NO:208	-0.2928	9.4654	1.27	12.65
miR-148b	miR-148bGSP2	CATGATCAGCTGGGCCAAGAACAAAGTTC SEQ ID NO:209	miR-148bRP	T+CA+GTGCATCACAG SEQ ID NO:210	-0.2982	10.417	0.24	2.44

Human Target micro RNA	Extension Primer Name	Extension Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Slope	Intercept	Background RNA input 50ug	5ug
iiR-149	miR-149GSP2	CATGATCAGCTGGGCCAAGAGGAGTGAAG SEQ ID NO:211	miR-149RP	T+CT+GGCTCCGTGTC SEQ ID NO:212	-0.2996	8.3392	2.15	21.50
iiR-150	miR-150GSP3	CATGATCAGCTGGGCCAAGACACTGGTA SEQ ID NO:213	miR-150RP	T+CT+CCCAACCCTTG SEQ ID NO:214	-0.2943	8.3945	0.06	0.56
miR-151	miR-151GSP2	CATGATCAGCTGGGCCAAGACCTCAAGGA SEQ ID NO:215	miR-151RP	A+CT+AGACTGAAGCTC SEQ ID NO:216	-0.2975	8.651	0.16	1.60
miR-152	miR-152GSP2	CATGATCAGCTGGGCCAAGACCCAAGTTC SEQ ID NO:217	miR-152RP	T+CA+GTGCATGACAG SEQ ID NO:218	-0.2741	8.7404	0.33	3.25
miR-153	miR-153GSP2	CATGATCAGCTGGGCCAAGATCACTTTTG SEQ ID NO:219	miR-153RP	TTG+CAT+AGTCACAAAA SEQ ID NO:220	-0.2723	9.5732	3.32	33.19
miR-154*	miR-154*GSP9#	CATGATCAGCTGGGCCAAGAAATAGGTCA SEQ ID NO:221	miR-154*RP2#	AATCA+TA+CACGGTTGAC SEQ ID NO:222	-0.3056	8.8502	0.07	0.74
miR-154	miR-154GSP9#	CATGATCAGCTGGGCCAAGACGAAGGCAA SEQ ID NO:223	miR-154RP3#	TA+GGTTA+TCCGTGTT SEQ ID NO:224	-0.3062	9.3947	0.10	0.96
miR-155	miR-155GSP8#	CATGATCAGCTGGGCCAAGACCCCTATC SEQ ID NO:225	miR-155RP2#	TT+AA+TGCTAATCGTGATA GG SEQ ID NO:226	-0.3201	8.474	5.49	54.91
miR-181a	miR-181aGSP9#	CATGATCAGCTGGGCCAAGAACTCACCGA SEQ ID NO:227	miR-181aRP2#	AA+CATT+CAACGCTGTC SEQ ID NO:228	-0.2919	7.968	1.70	17.05
miR-181c	miR-181cGSP9#	CATGATCAGCTGGGCCAAGAACTCACCGA SEQ ID NO:229	miR-181cRP2#	AA+CATT+CAACCTGTCG SEQ ID NO:230	-0.3102	7.9029	1.08	10.78
miR-182*	miR-182*GSP	CATGATCAGCTGGGCCAAGATAGTTGGCAA SEQ ID NO:231	miR-182*RP	T+GG+TTCTAGACTTGC SEQ ID NO:232	-0.2978	8.5876	4.25	42.47
miR-182	miR-182GSP2	CATGATCAGCTGGGCCAAGATGTGAGTTC SEQ ID NO:233	miR-182RP	TTT+GG+CAATGGTAG SEQ ID NO:234	-0.2863	9.0854	1.52	15.20

Human Target micro RNA	Extension Primer Name	Extension Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Slope	Intercept	Background RNA input 50ug	5ug
iR-183	miR-183GSP2	CATGATCAGCTGGGCCAAGACAGTGAATT SEQ ID NO:235	miR-183RP	T+AT+GGCACTGGTAG SEQ ID NO:236	-0.2774	9.9254	1.95	19.51
iR-184	miR-184GSP2	CATGATCAGCTGGGCCAAGAACCCTTATC SEQ ID NO:237	miR-184RP	T+GG+ACGGAGAACTG SEQ ID NO:238	-0.2906	7.9585	0.05	0.49
miR-186	miR-186GSP9#	CATGATCAGCTGGGCCAAGAAAGCCCAAA SEQ ID NO:239	miR-186RP3#	CA+AA+GAATT+CTCCTTTTGG SEQ ID NO:240	-0.2861	8.6152	0.32	3.18
miR-187	miR-187GSP	CATGATCAGCTGGGCCAAGACGGCTGCAAC SEQ ID NO:241	miR-187RP	T+CG+TGTCTTGTGTT SEQ ID NO:242	-0.2953	7.9329	1.23	12.31
miR-188	miR-188GSP	CATGATCAGCTGGGCCAAGAACCCTCCACC SEQ ID NO:243	miR-188RP	C+AT+CCCTTGCATGG SEQ ID NO:244	-0.2925	8.0782	8.49	84.92
miR-189	miR-189GSP2	CATGATCAGCTGGGCCAAGAACTGATATC SEQ ID NO:245	miR-189RP	G+TG+CCTACTGAGCT SEQ ID NO:246	-0.2981	8.8964	0.21	2.08
miR-190	miR-190GSP9#	CATGATCAGCTGGGCCAAGAACCTAATAT SEQ ID NO:247	miR-190RP4#	T+GA+TA+TGTTTGATATATTAG SEQ ID NO:248	-0.3317	9.8766	0.43	4.34
miR-191	miR-191GSP2	CATGATCAGCTGGGCCAAGAAGCTGCTTT SEQ ID NO:249	miR-191RP2	C+AA+CGGAATCCCAAAAG SEQ ID NO:250	-0.299	9.0317	0.41	4.07
miR-192	miR-192GSP2	CATGATCAGCTGGGCCAAGAGGCTGTCAA SEQ ID NO:251	miR-192RP	C+TGA+CCTATGAATTGAC SEQ ID NO:252	-0.2924	9.5012	1.10	10.98
miR-193	miR-193GSP9#	CATGATCAGCTGGGCCAAGACTGGGACTT SEQ ID NO:253	miR-193RP2#	AA+CT+GGCCTACAAAG SEQ ID NO:254	-0.3183	8.9942	0.17	1.72
miR-194	mir194GSP8#	CATGATCAGCTGGGCCAAGATCCACATG SEQ ID NO:255	mir194RP#	TG+TAA+CAGCAACTCCA SEQ ID NO:256	-0.3078	8.8045	0.37	3.69

Human Target micro RNA	Extension Primer Name	Extension Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Slope	Intercept	Background RNA input 50ug	5ug
iR-195	miR-195GSP9#	CATGATCAGCTGGGCCAAGAGCCAATATT SEQ ID NO:257	miR-195RP3#	T+AG+CAG+CACAGAAATA SEQ ID NO:258	-0.2955	10.213	0.76	7.58
...iR-196b	miR-196bGSP	CATGATCAGCTGGGCCAAGACCAACAACAG SEQ ID NO:259	miR-196bRP	TA+GGT+AGTTTCCTGT SEQ ID NO:260	-0.301	8.1641	1.47	14.66
miR-196a	miR-196aGSP	CATGATCAGCTGGGCCAAGACCAACAACAT SEQ ID NO:261	miR-196aRP	TA+GG+TAGTTTCATGTTG SEQ ID NO:262	-0.2932	8.0448	8.04	80.37
miR-197	miR-197GSP2	CATGATCAGCTGGGCCAAGAGCTGGGTGG SEQ ID NO:263	miR-197RP	TT+CA+CCACCTTCTC SEQ ID NO:264	-0.289	8.2822	0.71	7.10
miR-198	miR-198GSP3	CATGATCAGCTGGGCCAAGACCTATCTC SEQ ID NO:265	miR-198RP	G+GT+CCAGAGGGGAG SEQ ID NO:266	-0.2986	8.1359	0.31	3.15
miR-199a*	miR-199a*GSP2	CATGATCAGCTGGGCCAAGAAACCAATGT SEQ ID NO:267	miR-199a*RP	T+AC+AGTAGTCTGCAC SEQ ID NO:268	-0.3029	9.0509	0.25	2.52
miR-199a	miR-199aGSP2	CATGATCAGCTGGGCCAAGAGAACAGGTA SEQ ID NO:269	miR-199aRP	C+CC+AGTGTTCAGAC SEQ ID NO:270	-0.3187	9.2268	0.12	1.16
miR-199b	miR-199bGSP	CATGATCAGCTGGGCCAAGAGAACAGATAG SEQ ID NO:271	miR-199bRP	C+CC+AGTGTTTAGAC SEQ ID NO:272	-0.3165	9.3935	2.00	20.04
miR-200a	miR-200aGSP2	CATGATCAGCTGGGCCAAGAACATCGTTA SEQ ID NO:273	miR-200aRP	TAA+CAC+TGTCTGGT SEQ ID NO:274	-0.2754	9.1227	0.08	0.78
miR-200b	miR-200bGSP2	CATGATCAGCTGGGCCAAGAGTCATCATT SEQ ID NO:275	miR-200bRP	TAATA+CTG+CCTGGTAAT SEQ ID NO:276	-0.2935	8.5461	0.08	0.85
miR-202	miR-202 GSP10#	CATGATCAGCTGGGCCAAGATTTTCCCATG SEQ ID NO:277	miR-202RP#	A+GA+GGTATA+GGGCAT SEQ ID NO:278	-0.2684	9.056	0.25	2.48
miR-203	miR-203GSP2	CATGATCAGCTGGGCCAAGACTAGTGGTC SEQ ID NO:279	miR-203RP	G+TG+AAATGTTTAGGACC SEQ ID NO:280	-0.2852	8.1279	1.60	16.03

Human Target micro RNA	Extension Primer Name	Extension Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Slope	Intercept	Background RNA input 50ug	5ug
iR-204	miR-204GSP2	CATGATCAGCTGGGCCAAGAAGGCATAGG SEQ ID NO:281	miR-204RP	T+TC+CCTTTGTCATCC SEQ ID NO:282	-0.2925	8.7648	0.16	1.59
..iR-205	miR-205GSP	CATGATCAGCTGGGCCAAGACAGACTCCGG SEQ ID NO:283	miR-205RP	T+CCTT+CATTCCACC SEQ ID NO:284	-0.304	8.2407	9.21	92.15
miR-206	mir206GSP7#	CATGATCAGCTGGGCCAAGACCACACA SEQ ID NO:285	miR-206RP#	T+G+GAA+TGTAAGGAAGT GT SEQ ID NO:286	-0.2815	8.2206	0.29	2.86
miR-208	miR-208_GSP13#	CATGATCAGCTGGGCCAAGAACAAGCTTTTTGC SEQ ID NO:287	miR-208_RP4#	ATAA+GA+CG+AGCAAAAA G SEQ ID NO:288	-0.2072	7.9097	57.75	577.52
miR-210	miR-210GSP	CATGATCAGCTGGGCCAAGATCAGCCGCTG SEQ ID NO:289	miR-210RP	C+TG+TGCGTGTGACA SEQ ID NO:290	-0.2717	8.249	0.18	1.77
miR-211	miR-211GSP2	CATGATCAGCTGGGCCAAGAAGGCGAAGG SEQ ID NO:291	miR-211RP	T+TC+CCTTTGTCATCC SEQ ID NO:292	-0.2926	8.3106	0.10	1.00
miR-212	miR-212GSP9#	CATGATCAGCTGGGCCAAGAGGCCGTGAC SEQ ID NO:293	miR-212RP2#	T+AA+CAGTCTCCAGTCA SEQ ID NO:294	-0.2916	8.0745	0.59	5.86
miR-213	miR-213GSP	CATGATCAGCTGGGCCAAGAGGTACAATCA SEQ ID NO:295	miR-213RP	A+CC+ATCGACCGTTG SEQ ID NO:296	-0.2934	8.1848	2.96	29.59
miR-214	miR-214GSP	CATGATCAGCTGGGCCAAGACTGCCTGTCT SEQ ID NO:297	miR-214RP	A+CA+GCAGGCACAGA SEQ ID NO:298	-0.2947	7.82	0.84	8.44
miR-215	miR-215GSP2	CATGATCAGCTGGGCCAAGAGTCTGTCAA SEQ ID NO:299	miR-215RP	A+TGA+CCTATGAATTGAC SEQ ID NO:300	-0.2932	8.9273	1.51	15.05
miR-216	miR-216GSP9#	CATGATCAGCTGGGCCAAGACACAGTTGC SEQ ID NO:301	mir216RP#	TAA+TCT+CAGCTGGCA SEQ ID NO:302	-0.273	8.5829	0.95	9.50

Human Target micro RNA	Extension Primer Name	Extension Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Slope	Intercept	Background RNA input 50ug	5ug
iR-217	miR-217GSP2	CATGATCAGCTGGGCCAAGAATCCAATCA SEQ ID NO:303	miR-217RP2	T+AC+TGCATCAGGAACTGA SEQ ID NO:304	-0.3089	9.6502	0.07	0.71
iR-218	miR-218GSP2	CATGATCAGCTGGGCCAAGAACATGGTTA SEQ ID NO:305	miR-218RP	TTG+TGCTT+GATCTAAC SEQ ID NO:306	-0.2778	8.4363	1.00	10.05
miR-220	miR-220GSP	CATGATCAGCTGGGCCAAGAAAAGTGTCAG SEQ ID NO:307	miR-220RP	C+CA+CACCGTATCTG SEQ ID NO:308	-0.2755	9.0728	8.88	88.75
miR-221	miR-221GSP9#	CATGATCAGCTGGGCCAAGAGAAACCCAG SEQ ID NO:309	miR-221RP#	A+GC+TACATTGTCTGC SEQ ID NO:310	-0.2886	8.5743	0.12	1.17
miR-222	miR-222GSP8#	CATGATCAGCTGGGCCAAGAGAGACCCA SEQ ID NO:311	miR-222RP#	A+GC+TACATCTGGCT SEQ ID NO:312	-0.283	8.91	1.64	16.41
miR-223	miR-223GSP	CATGATCAGCTGGGCCAAGAGGGGTATTTG SEQ ID NO:313	miR-223RP	TG+TC+AGTTTGTCAAA SEQ ID NO:314	-0.2998	8.6669	0.94	9.44
miR-224	miR-224GSP8#	CATGATCAGCTGGGCCAAGATAAACGGA SEQ ID NO:315	miR-224RP2#	C+AAG+TCACTAGTGGTT SEQ ID NO:316	-0.2802	7.5575	0.56	5.63
miR-296	miR-296GSP9#	CATGATCAGCTGGGCCAAGAACAGGATTG SEQ ID NO:317	miR-296RP2#	A+GG+GCCCCCCCTCAA SEQ ID NO:318	-0.3178	8.3856	0.10	0.96
miR-299	miR-299GSP9#	CATGATCAGCTGGGCCAAGAATGTATGTG SEQ ID NO:319	miR-299RP#	T+GG+TTTACCGTCCC SEQ ID NO:320	-0.3155	7.9383	1.30	12.96
miR-301	miR-301GSP	CATGATCAGCTGGGCCAAGAGCTTTGACAA SEQ ID NO:321	miR-301RP	C+AG+TGCAATAGTATTGT SEQ ID NO:322	-0.2839	8.314	2.55	25.52
miR-302a*	miR-302a*GSP	CATGATCAGCTGGGCCAAGAAAAGCAAGTA SEQ ID NO:323	miR-302a*RP	TAAA+CG+TGGATGTAC SEQ ID NO:324	-0.2608	8.3921	0.04	0.41
miR-302a	miR-302aGSP	CATGATCAGCTGGGCCAAGATCACCAAAAC SEQ ID NO:325	miR-302aRP	T+AAG+TGCTTCCATGT SEQ ID NO:326	-0.2577	9.6657	2.17	21.67

Human Target micro RNA	Extension Primer Name	Extension Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Slope	Intercept	Background RNA input 50ug	5ug
iiR-02b*	miR-302b*GSP	CATGATCAGCTGGGCCAAGAAGAAAGCACT SEQ ID NO:327	miR-302b*RP	A+CTTTAA+CATGGAAGTG SEQ ID NO:328	-0.2702	8.5153	0.02	0.24
..iiR-302b	miR-302bGSP	CATGATCAGCTGGGCCAAGACTACTAAAAC SEQ ID NO:329	miR-302bRP	T+AAG+TGCTTCCATGT SEQ ID NO:330	-0.2398	9.1459	5.11	51.11
miR-302d	miR-302dGSP	CATGATCAGCTGGGCCAAGAACACTCAAAC SEQ ID NO:331	miR-302dRP	T+AAG+TGCTTCCATGT SEQ ID NO:332	-0.2368	8.5602	5.98	59.78
miR-302c*	miR-302c*_GSP9#	CATGATCAGCTGGGCCAAGACAGCAGGTA SEQ ID NO:333	miR-302c*_RP2#	TT+TAA+CAT+GGGGGTACC SEQ ID NO:334	-0.312	8.2904	0.33	3.28
miR-302c	miR-302cGSP9#	CATGATCAGCTGGGCCAAGACCACTGAAA SEQ ID NO:335	miR-302cRP5#	T+AAG+TGCTTCCATGTTTC A SEQ ID NO:336	-0.2945	8.381	14.28	142.76
miR-320	miR-320_GSP8#	CATGATCAGCTGGGCCAAGATTCGCCCT SEQ ID NO:337	miR-320_RP3#	AAAA+GCT+GGGTTGAGAG G SEQ ID NO:338	-0.2677	7.8956	6.73	67.29
miR-323	miR-323GSP	CATGATCAGCTGGGCCAAGAAGAGGTCGAC SEQ ID NO:339	miR-323RP	G+CA+CATTACACGGT SEQ ID NO:340	-0.2878	8.2546	0.19	1.92
miR-324-3p	miR-324-3pGSP	CATGATCAGCTGGGCCAAGACCAGCAGCAC SEQ ID NO:341	miR-324-3pRP	C+CA+CTGCCCCAGGT SEQ ID NO:342	-0.2698	8.5223	2.54	25.41
miR-324-5p	miR-324-5pGSP	CATGATCAGCTGGGCCAAGAACACCAATGC SEQ ID NO:343	miR-324-5pRP	C+GC+ATCCCCTAGGG SEQ ID NO:344	-0.2861	7.6865	0.06	0.62
miR-325	miR-325GSP	CATGATCAGCTGGGCCAAGAACACTTACTG SEQ ID NO:345	miR-325RP	C+CT+AGTAGGTGTCC SEQ ID NO:346	-0.2976	8.1925	0.01	0.14
miR-326	miR-326GSP	CATGATCAGCTGGGCCAAGACTGGAGGAAG SEQ ID NO:347	miR-326RP	C+CT+CTGGGCCCTTC SEQ ID NO:348	-0.2806	7.897	0.59	5.87

Human Target micro RNA	Extension Primer Name	Extension Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Slope	Intercept	Background RNA input 50ug	5ug
iR-328	miR-328GSP	CATGATCAGCTGGGCCAAGAACGGAAGGGC SEQ ID NO:349	miR-328RP	C+TG+GCCCTCTCTGC SEQ ID NO:350	-0.293	7.929	3.17	31.69
iR-330	miR-330GSP	CATGATCAGCTGGGCCAAGATCTCTGCAGG SEQ ID NO:351	miR-330RP	G+CA+AAGCACACGGC SEQ ID NO:352	-0.3009	7.7999	0.13	1.30
miR-331	miR-331GSP	CATGATCAGCTGGGCCAAGATTCTAGGATA SEQ ID NO:353	miR-331RP	G+CC+CCTGGGCCTAT SEQ ID NO:354	-0.2816	8.1643	0.45	4.54
miR-337	miR-337GSP	CATGATCAGCTGGGCCAAGAAAAGGCATCA SEQ ID NO:355	miR-337RP	T+CC+AGCTCCTATATG SEQ ID NO:356	-0.2968	8.7313	0.10	1.02
miR-338	miR-338GSP	CATGATCAGCTGGGCCAAGATCAACAAAAT SEQ ID NO:357	miR-338RP2	T+CC+AGCATCAGTGATTT SEQ ID NO:358	-0.2768	8.5618	0.52	5.17
miR-339	miR-339GSP9#	CATGATCAGCTGGGCCAAGATGAGCTCCT SEQ ID NO:359	miR-339RP2#	T+CC+CTGTCCTCCAGG SEQ ID NO:360	-0.303	8.4873	0.27	2.72
miR-340	miR-340GSP	CATGATCAGCTGGGCCAAGAGGCTATAAAG SEQ ID NO:361	miR-340RP	TC+CG+TCTCAGTTAC SEQ ID NO:362	-0.2846	9.6673	0.15	1.45
miR-342	miR-342GSP3	CATGATCAGCTGGGCCAAGAGACGGGTG SEQ ID NO:363	miR-342RP	T+CT+CACACAGAAATCG SEQ ID NO:364	-0.293	8.1553	4.69	46.85
miR-345	miR-345GSP	CATGATCAGCTGGGCCAAGAGCCCTGGACT SEQ ID NO:365	miR-345RP	T+GC+TGACTCCTAGT SEQ ID NO:366	-0.2909	8.468	0.04	0.40
miR-346	miR-346GSP	CATGATCAGCTGGGCCAAGAAGAGGCAGGC SEQ ID NO:367	miR-346RP	T+GT+CTGCCCGCATG SEQ ID NO:368	-0.2959	8.1958	0.25	2.54
miR-363	miR-363 GSP10#	CATGATCAGCTGGGCCAAGATACAGATGGA SEQ ID NO:369	miR-363RP#	AAT+TG+CAC+GGTATCC SEQ ID NO:370	-0.2362	8.9762	0.44	4.36
miR-367	miR-367GSP	CATGATCAGCTGGGCCAAGATCACCATTGC SEQ ID NO:371	miR-367RP	AAT+TG+CACTTTAGCAAT SEQ ID NO:372	-0.2819	8.6711	0.00	0.03

Human Target micro RNA	Extension Primer Name	Extension Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Slope	Intercept	Background RNA input 50ug	5ug
iiR-368	miR-368GSP	CATGATCAGCTGGGCCAAGAAAACGTGGAA SEQ ID NO:373	miR-368RP2	A+CATAGA+GGAAATTCCA C SEQ ID NO:374	-0.2953	8.0067	6.01	60.11
miR-370	miR-370GSP	CATGATCAGCTGGGCCAAGACCAGGTTCCA SEQ ID NO:375	miR-370RP	G+CC+TGCTGGGGTGG SEQ ID NO:376	-0.2825	8.3162	1.45	14.55
miR-371	miR-371GSP	CATGATCAGCTGGGCCAAGAACACTCAAAA SEQ ID NO:377	miR-371RP	G+TG+CCGCCATCTTT SEQ ID NO:378	-0.295	7.8812	2.51	25.12
miR-372	miR-372GSP	CATGATCAGCTGGGCCAAGAACGCTCAAAT SEQ ID NO:379	miR-372RP	A+AA+GTGCTGCGACA SEQ ID NO:380	-0.2984	8.9183	0.05	0.53
miR-373*	miR-373*GSP	CATGATCAGCTGGGCCAAGAGGAAAGCGCC SEQ ID NO:381	miR-373*RP	A+CT+CAAAATGGGGG SEQ ID NO:382	-0.2705	8.4513	0.20	1.99
miR-373	miR-373GSP	CATGATCAGCTGGGCCAAGAACACCCCAAA SEQ ID NO:383	miR-373RP2	GA+AG+TGCTTCGATTTTGG SEQ ID NO:384	-0.307	7.9056	9.13	91.32
miR-374	miR-374GSP2	CATGATCAGCTGGGCCAAGACACTTATCA SEQ ID NO:385	miR-374RP	TT+AT+AATA+CAACCTGAT AAG SEQ ID NO:386	-0.2655	9.3795	9.16	91.60
miR-375	miR-375GSP	CATGATCAGCTGGGCCAAGATCACGCGAGC SEQ ID NO:387	miR-375RP	TT+TG+TTCGTTCGGC SEQ ID NO:388	-0.3041	8.1181	0.09	0.90
miR-376b	miR-376b GSP8#	CATGATCAGCTGGGCCAAGAAACATGGA SEQ ID NO:389	miR-376bRP#	AT+CAT+AGA+GGAAAATCC A SEQ ID NO:390	-0.2934	9.0188	1.07	10.74
miR-378	miR-378GSP	CATGATCAGCTGGGCCAAGAACACAGGACC SEQ ID NO:391	miR-378RP	C+TC+CTGACTCCAGG SEQ ID NO:392	-0.2899	8.1467	0.07	0.73
miR-379	miR-379_GSP7#	CATGATCAGCTGGGCCAAGATACGTTC SEQ ID NO:393	miR-379RP2#	T+GGT+AGACTATGGAACG SEQ ID NO:394	-0.2902	8.2149	10.89	108.86

Human Target micro RNA	Extension Primer Name	Extension Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Slope	Intercept	Background RNA input 50ug	5ug
iR-380-)	miR-380-5pGSP	CATGATCAGCTGGGCCAAGAGCGCATGTTC SEQ ID NO:395	miR-380-5pRP	T+GGT+TGACCATAGA SEQ ID NO:396	-0.2462	9.4324	1.30	13.04
..iR-380-3p	miR-380-3pGSP	CATGATCAGCTGGGCCAAGAAAGATGTGGA SEQ ID NO:397	miR-380-3pRP	TA+TG+TAATATGGTCCACA SEQ ID NO:398	-0.3037	8.0356	3.69	36.89
miR-381	miR-381GSP2	CATGATCAGCTGGGCCAAGAACAGAGAGC SEQ ID NO:399	miR-381RP2	TATA+CAA+GGGCAAGCT SEQ ID NO:400	-0.3064	8.8704	1.72	17.16
miR-382	miR-382GSP	CATGATCAGCTGGGCCAAGACGAATCCACC SEQ ID NO:401	miR-382RP	G+AA+GTTGTTCGTGGT SEQ ID NO:402	-0.2803	7.6738	0.66	6.57
miR-383	miR-383GSP	CATGATCAGCTGGGCCAAGAAGCCACAATC SEQ ID NO:403	miR-383RP2	A+GATC+AGAAGGTGATTG T SEQ ID NO:404	-0.2866	8.1463	0.54	5.45
miR-410	miR-410 GSP9#	CATGATCAGCTGGGCCAAGAACAGGCCAT SEQ ID NO:405	miR-410RP#	AA+TA+TAA+CA+CAGATGG C SEQ ID NO:406	-0.2297	8.5166	4.27	42.71
miR-412	miR-412 GSP10#	CATGATCAGCTGGGCCAAGAACGGCTAGTG SEQ ID NO:407	miR-412RP#	A+CTT+CACCTGGTCCACTA SEQ ID NO:408	-0.3001	7.9099	4.24	42.37
miR-422a	miR-422aGSP	CATGATCAGCTGGGCCAAGAGGCCTTCTGA SEQ ID NO:409	miR-422aRP	C+TG+GACTTAGGGTC SEQ ID NO:410	-0.3079	9.3108	5.95	59.54
miR-422b	miR-422bGSP	CATGATCAGCTGGGCCAAGAGGCCTTCTGA SEQ ID NO:411	miR-422bRP	C+TG+GACTTGGAGTC SEQ ID NO:412	-0.2993	8.9437	4.86	48.56
miR-423	miR-423GSP	CATGATCAGCTGGGCCAAGACTGAGGGGCC SEQ ID NO:413	miR-423RP	A+GC+TCGGTCTGAGG SEQ ID NO:414	-0.3408	9.2274	6.06	60.62
miR-424	miR-424GSP#	CATGATCAGCTGGGCCAAGATTCAAAACAT SEQ ID NO:415	miR-424RP2#	C+AG+CAGCAATTCATGTTT T SEQ ID NO:416	-0.3569	9.3419	10.78	107.85

man rget icro NA	Extension Primer Name	Extension Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Slope	Intercept	Background RNA input 50ug	5ug
-425	miR-425GSP	CATGATCAGCTGGGCCAAGAGGCGGACACG SEQ ID NO:417	miR-425RP	A+TC+GGGAATGTCGT SEQ ID NO:418	-0.2932	7.9786	0.39	3.93
miR-429	miR-429_GSP11#	CATGATCAGCTGGGCCAAGAACGGTTTTACC SEQ ID NO:419	miR-429RP5#	T+AATAC+TG+TCTGGTAAA A SEQ ID NO:420	-0.2458	8.2805	16.21	162.12
miR-431	miR-431 GSP10#	CATGATCAGCTGGGCCAAGATGCATGACGG SEQ ID NO:421	miR-431RP#	T+GT+CTTGCAGGCCG SEQ ID NO:422	-0.3107	7.7127	7.00	70.05
miR-448	miR-448GSP	CATGATCAGCTGGGCCAAGAATGGGACATC SEQ ID NO:423	miR-448RP	TTG+CATA+TGTAGGATG SEQ ID NO:424	-0.3001	8.4969	0.12	1.16
miR-449	miR-449GSP10#	CATGATCAGCTGGGCCAAGAACCAGCTAAC SEQ ID NO:425	miR-449RP2#	T+GG+CAGTGTATTGTTAGC SEQ ID NO:426	-0.3225	8.4953	2.57	25.70
miR-450	miR-450GSP	CATGATCAGCTGGGCCAAGATATTAGGAAC SEQ ID NO:427	miR-450RP	TTTT+TG+CGATGTGTT SEQ ID NO:428	-0.2906	8.1404	0.48	4.82
miR-451	miR-451 GSP10#	CATGATCAGCTGGGCCAAGAAAACTCAGTA SEQ ID NO:429	miR-451RP#	AAA+CCG+TTA+CCATTACT GA SEQ ID NO:430	-0.2544	8.0291	1.73	17.35
let7a	let7a-GSP2#	CATGATCAGCTGGGCCAAGAAACTATAC SEQ ID NO:431	let7a-RP#	T+GA+GGTAGTAGGTTG SEQ ID NO:432	-0.3089	9.458	0.04	0.38
let7b	let7b-GSP2#	CATGATCAGCTGGGCCAAGAAACCACAC SEQ ID NO:433	let7b-RP#	T+GA+GGTAGTAGGTTG SEQ ID NO:432	-0.2978	7.9144	0.05	0.54
let7c	let7c-GSP2#	CATGATCAGCTGGGCCAAGAAACCATAC SEQ ID NO:434	let7c-RP#	T+GA+GGTAGTAGGTTG SEQ ID NO:432	-0.308	7.9854	0.01	0.14
let7d	let7d-GSP2#	CATGATCAGCTGGGCCAAGAACTATGCA SEQ ID NO:435	let7d-RP#	A+GA+GGTAGTAGGTTG SEQ ID NO:436	-0.3238	8.3359	0.06	0.57

Human Target micro RNA	Extension Primer Name	Extension Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Slope	Intercept	Background RNA input 50ug	5ug
:t7e	let7e-GSP2#	CATGATCAGCTGGGCCAAGAACTATACA SEQ ID NO:437	let7e-RP#	T+GA+GGTAGGAGGTTG SEQ ID NO:438	-0.3284	9.7594	0.22	2.20
..t7f	let7f-GSP2#	CATGATCAGCTGGGCCAAGAAACTATAC SEQ ID NO:439	let7f-RP#	T+GA+GGTAGTAGATTG SEQ ID NO:440	-0.2901	11.107	0.32	3.18
let7g	let7g-GSP2#	CATGATCAGCTGGGCCAAGAACTGTACA SEQ ID NO:441	let7g-RP#	T+GA+GGTAGTAGTTTG SEQ ID NO:442	-0.3469	9.8235	0.16	1.64
let7i	let7i-GSP2#	CATGATCAGCTGGGCCAAGAACAGCACA SEQ ID NO:443	let7i-RP#	T+GA+GGTAGTAGTTTG SEQ ID NO:444	-0.321	10.82	0.20	1.99
miR-377	miR-377GSP	CATGATCAGCTGGGCCAAGAACAAAAGTTG SEQ ID NO:445	miR-377RP2	AT+CA+CACAAAGGCAAC SEQ ID NO:446	-0.2979	10.612	13.45	134.48
miR-376a	miR-376a_GSP7	CATGATCAGCTGGGCCAAGAACGTGGA SEQ ID NO:447	miR-376a_RP5	AT+CAT+AGA+GGAAAATCC SEQ ID NO:448	-0.2938	10.045	63.00	630.00
miR-22	miR-22GSP	CATGATCAGCTGGGCCAAGAACAGTTCTTC SEQ ID NO:449	miR-22RP	A+AG+CTGCCAGTTGA SEQ ID NO:450	-0.2862	8.883	20.46	204.58
miR-200c	miR-200cGSP2	CATGATCAGCTGGGCCAAGACCATCATTA SEQ ID NO:451	miR-200cRP	T+AA+TACTGCCGGGT SEQ ID NO:452	-0.3094	11.5	15.99	159.91
miR-24	miR-24GSP	CATGATCAGCTGGGCCAAGACTGTTCCTGC SEQ ID NO:453	miR-24RP	T+GG+CTCAGTTCAGC SEQ ID NO:454	-0.3123	8.6824	24.34	243.38
miR-29cDNA	miR-29cGSP10	CATGATCAGCTGGGCCAAGAACCGATTTCA SEQ ID NO:455	miR-29cRP	T+AG+CACCATTTGAAAT SEQ ID NO:456	-0.2975	8.8441	23.22	232.17
miR-18	miR-18GSP	CATGATCAGCTGGGCCAAGATATCTGCACT SEQ ID NO:457	miR-18RP	T+AA+GGTGCATCTAGT SEQ ID NO:458	-0.3209	9.0999	14.90	149.01
miR-185	miR-185GSP	CATGATCAGCTGGGCCAAGAGAACTGCCTT SEQ ID NO:459	miR-185RP	T+GG+AGAGAAAGGCA SEQ ID NO:460	-0.3081	8.9289	15.73	157.32

nan 'get cro ᴺA	Extension Primer Name	Extension Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Slope	Intercept	Background RNA input 50ug	5ug
181b	miR-181bGSP8#	CATGATCAGCTGGGCCAAGACCCACCGA SEQ ID NO:461	miR-181bRP2#	AA+CATT+CATTGCTGTC SEQ ID NO:462	-0.3115	10.846	15.87	158.67
miR-128a	miR-128aGSP	CATGATCAGCTGGGCCAAGAAAAAGAGACC SEQ ID NO:161	miR-128anLRP	TCACAGTGAACCGGT SEQ ID NO: 494	approx. -0.2866	approx. 8.0867	approx. 0.16	approx. 1.60
miR-138	miR-138GSP2	CATGATCAGCTGGGCCAAGACGGCCTGAT SEQ ID NO:187	miR-138nLRP	AGCTGGTGTTGTGAA SEQ ID NO: 495	approx. -0.3023	approx. 9.0814	approx. 0.22	approx. 2.19
miR-143	miR-143GSP8#	CATGATCAGCTGGGCCAAGATGAGCTAC SEQ ID NO:197	miR-143nLRP	TGAGATGAAGCACTGT SEQ ID NO: 496	approx. -0.3008	approx. 9.2675	approx. 0.37	approx. 3.71
miR-150	miR-150GSP3	CATGATCAGCTGGGCCAAGACACTGGTA SEQ ID NO:213	miR-150nLRP	TCTCCCAACCCTTGTA SEQ ID NO: 497	approx. -0.2943	approx. 8.3945	approx. 0.06	approx. 0.56
miR-181a	miR-181aGSP9#	CATGATCAGCTGGGCCAAGAACTCACCGA SEQ ID NO:227	miR-181anLRP	AACATTCAACGCTGT SEQ ID NO: 498	approx. -0.2919	approx. 7.968	approx. 1.70	approx. 17.05
miR-194	mir194GSP8#	CATGATCAGCTGGGCCAAGATCCACATG SEQ ID NO:255	miR-194nLRP	TGTAACAGCAACTCCA SEQ ID NO: 499	approx. -0.3078	approx. 8.8045	approx. 0.37	approx. 3.69

EXAMPLE 4

This Example describes assays and primers designed for quantitative analysis of murine miNRA expression patterns.

Methods: The representative murine microRNA target templates described in TABLE 7 are publically available accessible on the World Wide Web at the Wellcome Trust Sanger Institute website in the "miRBase sequence database" as described in Griffith-Jones et al. (2004), *Nucleic Acids Research* 52:D1O9-D111 and and Griffith-Jones et al. (2006), *Nucleic Acids Research* 34: D140-D144. As indicated below in TABLE 7, the murine microRNA templates are either totally identical to the corresponding human microRNA templates, identical in the overlapping sequence with differing ends, or contain one or more base pair changes as compared to the human microRNA sequence. The murine microRNA templates that are identical or that have identical overlapping sequence to the corresponding human templates can be assayed using the same primer sets designed for the human microRNA templates, as indicated in TABLE 7. For the murine microRNA templates with one or more base pair changes in comparison to the corresponding human templates, primer sets have been designed specifically for detection of the murine microRNA, and these primers are provided in TABLE 7. The extension primer reaction and quantitative PCR reactions for detection of the murine microRNA templates may be carried out as described in EXAMPLE 3.

TABLE 7: Primers to detect murine microRNA target templates

Mouse Target microRNA:	Extension Primer Name	Extension Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Mouse microRNA as compared to Human microRNA
miR-1	miR1GSP10	CATGATCAGCTGGGCCAAGATACATACTTC SEQ ID NO:47	miR-1RP	T+G+GAA+TG+TAAAGAAGT SEQ ID NO:48	Identical
miR-7	miR-7GSP10	CATGATCAGCTGGGCCAAGAAACAAAATC SEQ ID NO: 486	miR-7_RP6	T+GGAA+GACTTGTGATTTT SEQ ID NO: 487	one or more base pairs differ
miR-9*	miR-9*GSP	CATGATCAGCTGGGCCAAGAACTTTCGGTT SEQ ID NO:51	miR-9*RP	TAAA+GCT+AGATAACCG SEQ ID NO:52	Identical overlapping sequence, ends differ
miR-10a	miR-10aGSP	CATGATCAGCTGGGCCAAGACACAAATTCG SEQ ID NO:53	miR-10aRP	T+AC+CCTGTAGATCCG SEQ ID NO:54	Identical
miR-10b	miR-10b_GSP11	CATGATCAGCTGGGCCAAGAACACAAATTC G SEQ ID NO: 492	miR-10b_RP2	C+CC+TGT+AGAACCGAAT SEQ ID NO: 493	one or more base pairs differ
miR-15a	miR-15aGSP	CATGATCAGCTGGGCCAAGACACAAACCAT SEQ ID NO:57	miR-15aRP	T+AG+CAGCACATAATG SEQ ID NO:58	Identical
miR-15b	miR-15bGSP2	CATGATCAGCTGGGCCAAGATGTAAACCA SEQ ID NO:59	miR-15bRP	T+AG+CAGCACATCAT SEQ ID NO:60	Identical
miR-16	miR-16GSP2	CATGATCAGCTGGGCCAAGACGCCAATAT SEQ ID NO:61	miR-16RP	T+AG+CAGCACGTAAA SEQ ID NO:62	Identical
miR-17-3p	miR-17-3pGSP	CATGATCAGCTGGGCCAAGAACAAGTGCCC SEQ ID NO: 463	miR-17-3pRP	A+CT+GCAGTGAGGGC SEQ ID NO: 464	one or more base pairs differ
miR-17-5p	miR-17-5pGSP2	CATGATCAGCTGGGCCAAGAACTACCTGC SEQ ID NO:65	miR-17-5pRP	C+AA+AGTGCTTACAGTG SEQ ID NO:66	Identical
miR-19a	miR-19aGSP2	CATGATCAGCTGGGCCAAGATCAGTTTTG SEQ ID NO:67	miR-19aRP	TG+TG+CAAATCTATGC SEQ ID NO:68	Identical
miR-19b	miR-19bGSP	CATGATCAGCTGGGCCAAGATCAGTTTTGC SEQ ID NO:69	miR-19bRP	TG+TG+CAAATCCATG SEQ ID NO:70	Identical

Mouse Target microRNA:	Extension Primer Name	Extension Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Mouse microRNA as compared to Human microRNA
miR-20	miR-20GSP3	CATGATCAGCTGGGCCAAGACTACCTGC SEQ ID NO:71	miR-20RP	T+AA+AGTGCTTATAGTGCA SEQ ID NO:72	Identical
miR-21	miR-21GSP2	CATGATCAGCTGGGCCAAGATCAACATCA SEQ ID NO: 73	miR-21RP	T+AG+CTTATCAGACTGATG SEQ ID NO:74	Identical
miR-23a	miR-23aGSP	CATGATCAGCTGGGCCAAGAGGAAATCCCT SEQ ID NO:75	miR-23aRP	A+TC+ACATTGCCAGG SEQ ID NO:76	Identical
miR-23b	miR-23bGSP	CATGATCAGCTGGGCCAAGAGGTAATCCCT SEQ ID NO:77	miR-23bRP	A+TC+ACATTGCCAGG SEQ ID NO:78	Identical
miR-24	miR-24P5	CATGATCAGCTGGGCCAAGACTGTTCCTGC TG SEQ ID NO: 7	miR24-1,2R	TGG+CTCAGTTCAGC SEQ ID NO: 19	Identical
miR-25	miR-25GSP	CATGATCAGCTGGGCCAAGATCAGACCGAG SEQ ID NO:79	miR-25RP	C+AT+TGCACTTGTCTC SEQ ID NO:80	Identical
miR-26a	miR-26aGSP9	CATGATCAGCTGGGCCAAGAGCCTATCCT SEQ ID NO:81	miR-26aRP2	TT+CA+AGTAATCCAGGAT SEQ ID NO:82	Identical
miR-26b	miR-26bGSP9	CATGATCAGCTGGGCCAAGAAACCTATCC SEQ ID NO:83	miR-26bRP2	TT+CA+AGT+AATTCAGGAT SEQ ID NO:84	Identical
miR-27a	miR-27aGSP	CATGATCAGCTGGGCCAAGAGCGGAACTTA SEQ ID NO:85	miR-27aRP	TT+CA+CAGTGGCTAA SEQ ID NO:86	Identical
miR-27b	miR-27bGSP	CATGATCAGCTGGGCCAAGAGCAGAACTTA SEQ ID NO:87	miR-27bRP	TT+CA+CAGTGGCTAA SEQ ID NO:88	Identical
miR-28	miR-28GSP	CATGATCAGCTGGGCCAAGACTCAATAGAC SEQ ID NO:89	miR-28RP	A+AG+GAGCTCACAGT SEQ ID NO:90	Identical
miR-29a	miR-29aGSP8	CATGATCAGCTGGGCCAAGAAACCGATT SEQ ID NO:91	miR-29aRP2	T+AG+CACCATCTGAAAT SEQ ID NO:92	Identical
miR-29b	miR-29bGSP2	CATGATCAGCTGGGCCAAGAAACACTGAT SEQ ID NO:93	miR-29bRP2	T+AG+CACCATTTGAAATCAG SEQ ID NO:94	Identical

Mouse Target microRNA:	Extension Primer Name	Extension Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Mouse microRNA as compared to Human microRNA
miR-30a-5p	miR-30a-5pGSP	CATGATCAGCTGGGCCAAGACTTCCAGTCG SEQ ID NO:95	miR-30a-5pRP	T+GT+AAACATCCTCGAC SEQ ID NO:96	Identical
miR-30b	miR-30bGSP	CATGATCAGCTGGGCCAAGAAGCTGAGTGT SEQ ID NO:97	miR-30bRP	TGT+AAA+CATCCTACACT SEQ ID NO:98	Identical
miR-30c	miR-30cGSP	CATGATCAGCTGGGCCAAGAGCTGAGAGTG SEQ ID NO:99	miR-30cRP	TGT+AAA+CATCCTACACT SEQ ID NO:100	Identical
miR-30d	miR-30dGSP	CATGATCAGCTGGGCCAAGACTTCCAGTCG SEQ ID NO:101	miR-30dRP	T+GTAAA+CATCCCCG SEQ ID NO:102	Identical
miR-30e-3p	miR-30e-3pGSP9	CATGATCAGCTGGGCCAAGAGCTGTAAAC SEQ ID NO:103	miR-30e-3pRP5	CTTT+CAGT+CGGATGTTT SEQ ID NO:104	Identical
miR-31	miR-31GSP	CATGATCAGCTGGGCCAAGACAGCTATGCC SEQ ID NO:107	miR-31RP	G+GC+AAGATGCTGGC SEQ ID NO:108	Identical overlapping sequence, ends differ
miR-32	miR-32GSP	CATGATCAGCTGGGCCAAGAGCAACTTAGT SEQ ID NO:109	miR-32RP	TATTG+CA+CATTACTAAG SEQ ID NO:110	Identical
miR-33	miR-33GSP2	CATGATCAGCTGGGCCAAGACAATGCAAC SEQ ID NO:111	miR-33RP	G+TG+CATTGTAGTTGC SEQ ID NO:112	Identical
miR-34a	miR-34aGSP	CATGATCAGCTGGGCCAAGAAACAACCAGC SEQ ID NO:113	miR-34aRP	T+GG+CAGTGTCTTAG SEQ ID NO:114	Identical
miR-34b	miR-34bGSP	CATGATCAGCTGGGCCAAGACAATCAGCTA SEQ ID NO: 115	miR-34bRP	TA+GG+CAGTGTAATT SEQ ID NO: 482	one or more base pairs differ
miR-34c	miR-34cGSP	CATGATCAGCTGGGCCAAGAGCAATCAGCT SEQ ID NO:117	miR-34cRP	A+GG+CAGTGTAGTTA SEQ ID NO:118	Identical
miR-92	miR-92GSP	CATGATCAGCTGGGCCAAGACAGGCCGGGA SEQ ID NO:119	miR-92RP	T+AT+TGCACTTGTCCC SEQ ID NO:120	Identical
miR-93	miR-93GSP	CATGATCAGCTGGGCCAAGACTACCTGCAC SEQ ID NO:121	miR-93RP	AA+AG+TGCTGTTCGT SEQ ID NO:122	Identical overlapping sequence, ends differ
miR-96 .	miR-96GSP	CATGATCAGCTGGGCCAAGAGCAAAAATGT SEQ ID NO:125	miR-96RP	T+TT+GGCACTAGCAC SEQ ID NO:126	Identical overlapping sequence, ends differ

Mouse Target microRNA:	Extension Primer Name	Extension Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Mouse microRNA as compared to Human microRNA
miR-98	miR-98GSP	CATGATCAGCTGGGCCAAGAAACAATACAA SEQ ID NO:127	miR-98RP	TGA+GGT+AGTAAGTTG SEQ ID NO:128	Identical
miR-99a	miR-99aGSP	CATGATCAGCTGGGCCAAGACACAAGATCG SEQ ID NO:129	miR-99aRP	A+AC+CCGTAGATCCG SEQ ID NO:130	Identical overlapping sequence, ends differ
miR-99b	miR-99bGSP	CATGATCAGCTGGGCCAAGACGCAAGGTCG SEQ ID NO:131	miR-99bRP	C+AC+CCGTAGAACCG SEQ ID NO:132	Identical
miR-100	miR-100GSP	CATGATCAGCTGGGCCAAGACACAAGTTCG SEQ ID NO:133	miR-100RP	A+AC+CCGTAGATCCG SEQ ID NO:134	Identical
miR-101	miR-101GSP	CATGATCAGCTGGGCCAAGACTTCAGTTAT SEQ ID NO:135	miR-101RP	TA+CAG+TACTGTGATAACT SEQ ID NO:136	Identical
miR-103	miR-103GSP	CATGATCAGCTGGGCCAAGATCATAGCCCT SEQ ID NO:137	miR-103RP	A+GC+AGCATTGTACA SEQ ID NO:138	Identical
miR-106a	miR-106aGSP	CATGATCAGCTGGGCCAAGATACCTGCAC SEQ ID NO: 472	miR-106aRP	CAA+AG+TGCTAACAGTG SEQ ID NO: 473	one or more base pairs differ
miR-106b	miR-106bGSP	CATGATCAGCTGGGCCAAGAATCTGCACTG SEQ ID NO:143	miR-106bRP	T+AAAG+TGCTGACAGT SEQ ID NO:144	Identical
miR-107	miR-107GSP8	CATGATCAGCTGGGCCAAGATGATAGCC SEQ ID NO:145	miR-107RP2	A+GC+AGCATTGTACAG SEQ ID NO:146	Identical
miR-122a	miR-122aGSP	CATGATCAGCTGGGCCAAGAACAAACACCA SEQ ID NO:147	miR-122aRP	T+GG+AGTGTGACAAT SEQ ID NO:148	Identical
miR-124a	miR-124aGSP	CATGATCAGCTGGGCCAAGATGGCATTCAC SEQ ID NO:149	miR-124aRP	T+TA+AGGCACGCGGT SEQ ID NO:150	Identical overlapping sequence, ends differ
miR-125a	miR-125aGSP	CATGATCAGCTGGGCCAAGACACAGGTTAA SEQ ID NO:151	miR-125aRP	T+CC+CTGAGACCCTT SEQ ID NO:152	Identical
miR-125b	miR-125bGSP	CATGATCAGCTGGGCCAAGATCACAAGTTA SEQ ID NO:153	miR-125bRP	T+CC+CTGAGACCCTA SEQ ID NO:154	Identical
miR-126	miR-126GSP	CATGATCAGCTGGGCCAAGAGCATTATTAC SEQ ID NO:155	miR-126RP	T+CG+TACCGTGAGTA SEQ ID NO:156	Identical

Mouse Target microRNA:	Extension Primer Name	Extension Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Mouse microRNA as compared to Human microRNA
miR-126*	miR-126*GSP3	CATGATCAGCTGGGCCAAGACGCGTACC SEQ ID NO:157	miR-126*RP	C+ATT+ATTA+CTTTTGGTACG SEQ ID NO:158	Identical
miR-127	miR-127GSP	CATGATCAGCTGGGCCAAGAAGCCAAGCTC SEQ ID NO:159	miR-127RP	T+CG+GATCCGTCTGA SEQ ID NO:160	Identical overlapping sequence, ends differ
miR-128a	miR-128aGSP	CATGATCAGCTGGGCCAAGAAAAAGAGACC SEQ ID NO:161	miR-128aRP	T+CA+CAGTGAACCGG SEQ ID NO:162	Identical
miR-128b	miR-128bGSP	CATGATCAGCTGGGCCAAGAGAAAGAGACC SEQ ID NO:163	miR-128bRP	T+CA+CAGTGAACCGG SEQ ID NO:164	Identical
miR-130a	miR-130aGSP	CATGATCAGCTGGGCCAAGAATGCCCTTTT SEQ ID NO:167	miR-130aRP	C+AG+TGCAATGTTAAAAG SEQ ID NO:168	Identical
miR-130b	miR-130bGSP	CATGATCAGCTGGGCCAAGAATGCCCTTTC SEQ ID NO:169	miR-130bRP	C+AG+TGCAATGATGA SEQ ID NO:170	Identical
miR-132	miR-132GSP	CATGATCAGCTGGGCCAAGACGACCATGGC SEQ ID NO:171	miR-132RP	T+AA+CAGTCTACAGCC SEQ ID NO:172	Identical
miR-133a	miR-133aGSP	CATGATCAGCTGGGCCAAGAACAGCTGGTT SEQ ID NO:173	miR-133aRP	T+TG+GTCCCCTTCAA SEQ ID NO:174	Identical
miR-133b	miR-133bGSP	CATGATCAGCTGGGCCAAGATAGCTGGTTG SEQ ID NO:175	miR-133bRP	T+TG+GTCCCCTTCAA SEQ ID NO:176	Identical
miR-134	miR-134GSP	CATGATCAGCTGGGCCAAGACCCTCTGGTC SEQ ID NO:177	miR-134RP	T+GT+GACTGGTTGAC SEQ ID NO:178	Identical overlapping sequence, ends differ
miR-135a	miR-135aGSP	CATGATCAGCTGGGCCAAGATCACATAGGA SEQ ID NO:179	miR-135aRP	T+AT+GGCTTTTTATTCCT SEQ ID NO:180	Identical
miR-135b	miR-135bGSP	CATGATCAGCTGGGCCAAGACACATAGGAA SEQ ID NO:181	miR-135bRP	T+AT+GGCTTTTCATTCC SEQ ID NO:182	Identical
miR-136	miR-136GSP	CATGATCAGCTGGGCCAAGATCCATCATCA SEQ ID NO:183	miR-136RP	A+CT+CCATTTGTTTTGATG SEQ ID NO:184	Identical
miR-137	miR-137GSP	CATGATCAGCTGGGCCAAGACTACGCGTAT SEQ ID NO:185	miR-137RP	T+AT+TGCTTAAGAATACGC SEQ ID NO:186	Identical overlapping sequence, ends differ

Mouse Target microRNA:	Extension Primer Name	Extension Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Mouse microRNA as compared to Human microRNA
miR-138	miR-138GSP2	CATGATCAGCTGGGCCAAGACGGCCTGAT SEQ ID NO:187	miR-138RP	A+GC+TGGTGTTGTGA SEQ ID NO:188	Identical
miR-139	miR-139GSP	CATGATCAGCTGGGCCAAGAAGACACGTGC SEQ ID NO:189	miR-139RP	T+CT+ACAGTGCACGT SEQ ID NO:190	Identical
miR-140	miR-140GSP	CATGATCAGCTGGGCCAAGACTACCATAGG SEQ ID NO:191	miR-140RP	A+GT+GGTTTTACCCT SEQ ID NO:192	Identical overlapping sequence, ends differ
miR-141	miR-141GSP9	CATGATCAGCTGGGCCAAGACCATCTTTA SEQ ID NO:193	miR-141RP2	TAA+CAC+TGTCTGGTAA SEQ ID NO:194	Identical
miR-142-3p	miR-142-3pGSP3	CATGATCAGCTGGGCCAAGATCCATAAA SEQ ID NO:195	miR-142-3pRP	TGT+AG+TGTTTCCTACT SEQ ID NO:196	Identical overlapping sequence, ends differ
miR-143	miR-143GSP8	CATGATCAGCTGGGCCAAGATGAGCTAC SEQ ID NO:197	miR-143RP2	T+GA+GATGAAGCACTG SEQ ID NO:198	Identical
miR-144	miR-144GSP2	CATGATCAGCTGGGCCAAGACTAGTACAT SEQ ID NO:199	miR-144RP	TA+CA+GTAT+AGATGATG SEQ ID NO:200	Identical
miR-145	miR-145GSP2	CATGATCAGCTGGGCCAAGAAAGGGATTC SEQ ID NO:201	miR-145RP	G+TC+CAGTTTTCCCA SEQ ID NO:202	Identical
miR-146	miR-146GSP3	CATGATCAGCTGGGCCAAGAAACCCATG SEQ ID NO:203	miR-146RP	T+GA+GAACTGAATTCCA SEQ ID NO:204	Identical
miR-148a	miR-148aGSP2	CATGATCAGCTGGGCCAAGAACAAAGTTC SEQ ID NO:207	miR-148aRP2	T+CA+GTGCACTACAGAACT SEQ ID NO:208	Identical
miR-148b	miR-148bGSP2	CATGATCAGCTGGGCCAAGAACAAAGTTC SEQ ID NO:209	miR-148bRP	T+CA+GTGCATCACAG SEQ ID NO:210	Identical
miR-149	miR-149GSP2	CATGATCAGCTGGGCCAAGAGGAGTGAAG SEQ ID NO:211	miR-149RP	T+CT+GGCTCCGTGTC SEQ ID NO:212	Identical
miR-150	miR-150GSP3	CATGATCAGCTGGGCCAAGACACTGGTA SEQ ID NO:213	miR-150RP	T+CT+CCCAACCCTTG SEQ ID NO:214	Identical
miR-151	miR-151GSP2	CATGATCAGCTGGGCCAAGACCTCAAGGA SEQ ID NO: 215	miR-151RP	A+CT+AGACTGAGGCTC SEQ ID NO: 477	one or more base pairs differ

Mouse Target microRNA:	Extension Primer Name	Extension Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Mouse microRNA as compared to Human microRNA
miR-152	miR-152GSP2	CATGATCAGCTGGGCCAAGACCCAAGTTC SEQ ID NO:217	miR-152RP	T+CA+GTGCATGACAG SEQ ID NO:218	Identical
miR-153	miR-153GSP2	CATGATCAGCTGGGCCAAGATCACTTTTG SEQ ID NO:219	miR-153RP	TTG+CAT+AGTCACAAAA SEQ ID NO:220	Identical overlapping sequence, ends differ
miR-154	miR-154GSP9	CATGATCAGCTGGGCCAAGACGAAGGCAA SEQ ID NO:223	miR-154RP3	TA+GGTTA+TCCGTGTT SEQ ID NO:224	Identical
miR-155	miR-155GSP8	CATGATCAGCTGGGCCAAGACCCCTATC SEQ ID NO: 225	miR-155RP2	TT+AA+TGCTAATTGTGATAGG SEQ ID NO: 489	one or more base pairs differ
miR-181a	miR-181aGSP9	CATGATCAGCTGGGCCAAGAACTCACCGA SEQ ID NO:227	miR-181aRP2	AA+CATT+CAACGCTGTC SEQ ID NO:228	Identical
miR-181c	miR-181cGSP9	CATGATCAGCTGGGCCAAGAACTCACCGA SEQ ID NO:229	miR-181cRP2	AA+CATT+CAACCTGTCG SEQ ID NO:230	Identical
miR-182	miR-182*GSP	CATGATCAGCTGGGCCAAGATAGTTGGCAA SEQ ID NO:231	miR-182*RP	T+GG+TTCTAGACTTGC SEQ ID NO:232	Identical
miR-183	miR-183GSP2	CATGATCAGCTGGGCCAAGACAGTGAATT SEQ ID NO:235	miR-183RP	T+AT+GGCACTGGTAG SEQ ID NO:236	Identical
miR-184	miR-184GSP2	CATGATCAGCTGGGCCAAGAACCCTTATC SEQ ID NO:237	miR-184RP	T+GG+ACGGAGAACTG SEQ ID NO:238	Identical
miR-186	miR-186GSP9	CATGATCAGCTGGGCCAAGAAAGCCCAAA SEQ ID NO:239	miR-186RP3	CA+AA+GAATT+CTCCTTTTGG SEQ ID NO:240	Identical
miR-187	miR-187GSP	CATGATCAGCTGGGCCAAGACGGCTGCAAC SEQ ID NO:241	miR-187RP	T+CG+TGTCTTGTGTT SEQ ID NO:242	Identical overlapping sequence, ends differ
miR-188	miR-188GSP	CATGATCAGCTGGGCCAAGAACCCTCCACC SEQ ID NO:243	miR-188RP	C+AT+CCCTTGCATGG SEQ ID NO:244	Identical
miR-189	miR-189GSP2	CATGATCAGCTGGGCCAAGAACTGATATC SEQ ID NO:245	miR-189RP	G+TG+CCTACTGAGCT SEQ ID NO:246	Identical
miR-190	miR-190GSP9	CATGATCAGCTGGGCCAAGAACCTAATAT SEQ ID NO:247	miR-190RP4	T+GA+TA+TGTTTGATATATTAG SEQ ID NO:248	Identical

Mouse Target microRNA:	Extension Primer Name	Extension Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Mouse microRNA as compared to Human microRNA
miR-191	miR-191GSP2	CATGATCAGCTGGGCCAAGAAGCTGCTTT SEQ ID NO:249	miR-191RP2	C+AA+CGGAATCCCAAAAG SEQ ID NO:250	Identical
miR-192	miR-192GSP2	CATGATCAGCTGGGCCAAGAGGCTGTCAA SEQ ID NO:251	miR-192RP	C+TGA+CCTATGAATTGAC SEQ ID NO:252	Identical overlapping sequence, ends differ
miR-193	miR-193GSP9	CATGATCAGCTGGGCCAAGACTGGGACTT SEQ ID NO:253	miR-193RP2	AA+CT+GGCCTACAAAG SEQ ID NO:254	Identical
miR-194	mir194GSP8	CATGATCAGCTGGGCCAAGATCCACATG SEQ ID NO:255	mir194RP	TG+TAA+CAGCAACTCCA SEQ ID NO:256	Identical
miR-195	miR-195GSP9	CATGATCAGCTGGGCCAAGAGCCAATATT SEQ ID NO:257	miR-195RP3	T+AG+CAG+CACAGAAATA SEQ ID NO:258	Identical
miR-196a	*miR-196aGSP*	CATGATCAGCTGGGCCAAGACCAACAACAT SEQ ID NO:261	miR-196aRP	TA+GG+TAGTTTCATGTTG SEQ ID NO:262	*Identical*
miR-196b	miR-196bGSP	CATGATCAGCTGGGCCAAGACCAACAACAG SEQ ID NO:259	miR-196bRP	TA+GGT+AGTTTCCTGT SEQ ID NO:260	Identical
miR-199a*	miR-199a*GSP2	CATGATCAGCTGGGCCAAGAAACCAATGT SEQ ID NO:267	miR-199a*RP	T+AC+AGTAGTCTGCAC SEQ ID NO:268	Identical
miR-199a	miR-199aGSP2	CATGATCAGCTGGGCCAAGAGAACAGGTA SEQ ID NO:269	miR-199aRP	C+CC+AGTGTTCAGAC SEQ ID NO:270	Identical
miR-199b	miR-199bGSP	CATGATCAGCTGGGCCAAGAGAACAGGTAG SEQ ID NO: 475	miR-199bRP	C+CC+AGTGTTTAGAC SEQ ID NO: 272	one or more base pairs differ
miR-200a	miR-200aGSP2	CATGATCAGCTGGGCCAAGAACATCGTTA SEQ ID NO:273	miR-200aRP	TAA+CAC+TGTCTGGT SEQ ID NO:274	Identical
miR-200b	miR-200bGSP2	CATGATCAGCTGGGCCAAGAGTCATCATT SEQ ID NO:275	miR-200bRP	TAATA+CTG+CCTGGTAAT SEQ ID NO:276	Identical
miR-203	miR-203GSP2	CATGATCAGCTGGGCCAAGACTAGTGGTC SEQ ID NO:279	miR-203RP	G+TG+AAATGTTTAGGACC SEQ ID NO:280	Identical overlapping sequence, ends differ
miR-204	miR-204GSP2	CATGATCAGCTGGGCCAAGAAGGCATAGG SEQ ID NO:281	miR-204RP	T+TC+CCTTTGTCATCC SEQ ID NO:282	Identical overlapping sequence, ends differ

Mouse Target microRNA:	Extension Primer Name	Extension Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Mouse microRNA as compared to Human microRNA
miR-205	miR-205GSP	CATGATCAGCTGGGCCAAGACAGACTCCGG SEQ ID NO:283	miR-205RP	T+CCTT+CATTCCACC SEQ ID NO:284	Identical
miR-206	mir206GSP7	CATGATCAGCTGGGCCAAGACCACACA SEQ ID NO:285	miR-206RP	T+G+GAA+TGTAAGGAAGTGT SEQ ID NO:286	Identical
miR-208	miR-208_GSP13	CATGATCAGCTGGGCCAAGAACAAGCTTTT TGC SEQ ID NO:287	miR-208_RP4	ATAA+GA+CG+AGCAAAAAG SEQ ID NO:288	Identical
miR-210	miR-210GSP	CATGATCAGCTGGGCCAAGATCAGCCGCTG SEQ ID NO:289	miR-210RP	C+TG+TGCGTGTGACA SEQ ID NO:290	Identical
miR-211	miR-211GSP2	CATGATCAGCTGGGCCAAGAAGGCAAAGG SEQ ID NO: 491	miR-211RP	T+TC+CCTTTGTCATCC SEQ ID NO: 292	one or more base pairs differ
miR-212	miR-212GSP9	CATGATCAGCTGGGCCAAGAGGCCGTGAC SEQ ID NO:293	miR-212RP2	T+AA+CAGTCTCCAGTCA SEQ ID NO:294	Identical
miR-213	miR-213GSP	CATGATCAGCTGGGCCAAGAGGTACAATCA SEQ ID NO:295	miR-213RP	A+CC+ATCGACCGTTG SEQ ID NO:296	Identical
miR-214	miR-214GSP	CATGATCAGCTGGGCCAAGACTGCCTGTCT SEQ ID NO:297	miR-214RP	A+CA+GCAGGCACAGA SEQ ID NO:298	Identical
miR-215	miR-215GSP2	CATGATCAGCTGGGCCAAGAGTCTGTCAA SEQ ID NO: 299	miR-215RP	A+TGA+CCTATGATTTGAC SEQ ID NO: 469	one or more base pairs differ
miR-216	miR-216GSP9	CATGATCAGCTGGGCCAAGACACAGTTGC SEQ ID NO:301	mir216RP	TAA+TCT+CAGCTGGCA SEQ ID NO:302	Identical
miR-217	miR-217GSP2	CATGATCAGCTGGGCCAAGAATCCAGTCA SEQ ID NO:481	miR-217RP2	T+AC+TGCATCAGGAACTGA SEQ ID NO: 304	one or more base pairs differ
miR-218	miR-218GSP2	CATGATCAGCTGGGCCAAGAACATGGTTA SEQ ID NO:305	miR-218RP	TTG+TGCTT+GATCTAAC SEQ ID NO:306	Identical
miR-221	miR-221GSP9	CATGATCAGCTGGGCCAAGAGAAACCCAG SEQ ID NO:309	miR-221RP	A+GC+TACATTGTCTGC SEQ ID NO:310	Identical overlapping sequence, ends differ

Mouse Target microRNA:	Extension Primer Name	Extension Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Mouse microRNA as compared to Human microRNA
miR-222	miR-222GSP8	CATGATCAGCTGGGCCAAGAGAGACCCA SEQ ID NO:311	miR-222RP	A+GC+TACATCTGGCT SEQ ID NO:312	Identical
miR-223	miR-223GSP	CATGATCAGCTGGGCCAAGAGGGGTATTTG SEQ ID NO:313	miR-223RP	TG+TC+AGTTTGTCAAA SEQ ID NO:314	Identical
miR-224	miR-224GSP8	CATGATCAGCTGGGCCAAGATAAACGGA SEQ ID NO:315	miR-224RP2	C+AAG+TCACTAGTGGTT SEQ ID NO:316	Identical overlapping sequence, ends differ
miR-296	miR-296GSP9	CATGATCAGCTGGGCCAAGAACAGGATTG SEQ ID NO:317	miR-296RP2	A+GG+GCCCCCCCTCAA SEQ ID NO:318	Identical
miR-299	miR-299GSP9	CATGATCAGCTGGGCCAAGAATGTATGTG SEQ ID NO:319	miR-299RP	T+GG+TTTACCGTCCC SEQ ID NO:320	Identical
miR-301	miR-301GSP	CATGATCAGCTGGGCCAAGAGCTTTGACAA SEQ ID NO:321	miR-301RP	C+AG+TGCAATAGTATTGT SEQ ID NO:322	Identical
miR-302a	miR-302aGSP	CATGATCAGCTGGGCCAAGATCACCAAAAC SEQ ID NO:325	miR-302aRP	T+AAG+TGCTTCCATGT SEQ ID NO:326	Identical
miR-320	miR-320_GSP8	CATGATCAGCTGGGCCAAGATTCGCCCT SEQ ID NO:337	miR-320_RP3	AAAA+GCT+GGGTTGAGAGG SEQ ID NO:338	Identical
miR-323	miR-323GSP	CATGATCAGCTGGGCCAAGAAGAGGTCGAC SEQ ID NO:339	miR-323RP	G+CA+CATTACACGGT SEQ ID NO:340	Identical
miR-324-3p	miR-324-3pGSP	CATGATCAGCTGGGCCAAGACCAGCAGCAC SEQ ID NO:341	miR-324-3pRP	C+CA+CTGCCCCAGGT SEQ ID NO:342	Identical
miR-324-5p	miR-324-5pGSP	CATGATCAGCTGGGCCAAGAACACCAATGC SEQ ID NO:343	miR-324-5pRP	C+GC+ATCCCCTAGGG SEQ ID NO:344	Identical overlapping sequence, ends differ
miR-325	miR-325GSP	CATGATCAGCTGGGCCAAGAACACTTACTG SEQ ID NO: 345	miR-325RP	C+CT+AGTAGGTGCTC SEQ ID NO: 476	one or more base pairs differ
miR-326	miR-326GSP	CATGATCAGCTGGGCCAAGACTGGAGGAAG SEQ ID NO:347	miR-326RP	C+CT+CTGGGCCCTTC SEQ ID NO:348	Identical overlapping sequence, ends differ
miR-328	miR-328GSP	CATGATCAGCTGGGCCAAGAACGGAAGGGC SEQ ID NO:349	miR-328RP	C+TG+GCCCTCTCTGC SEQ ID NO:350	Identical

Mouse Target microRNA:	Extension Primer Name	Extension Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Mouse microRNA as compared to Human microRNA
miR-330	miR-330GSP	CATGATCAGCTGGGCCAAGATCTCTGCAGG SEQ ID NO: 351	miR-330RP	G+CA+AAGCACAGGGC SEQ ID NO: 478	one or more base pairs differ
miR-331	miR-331GSP	CATGATCAGCTGGGCCAAGATTCTAGGATA SEQ ID NO:353	miR-331RP	G+CC+CCTGGGCCTAT SEQ ID NO:354	Identical
miR-337	miR-337GSP	CATGATCAGCTGGGCCAAGAAAAGGCATCA SEQ ID NO:355	miR-337RP	T+TC+AGCTCCTATATG SEQ ID NO: 490	one or more base pairs differ
miR-338	miR-338GSP	CATGATCAGCTGGGCCAAGATCAACAAAAT SEQ ID NO:357	miR-338RP2	T+CC+AGCATCAGTGATTT SEQ ID NO:358	Identical
miR-339	miR-339GSP9	CATGATCAGCTGGGCCAAGATGAGCTCCT SEQ ID NO:359	miR-339RP2	T+CC+CTGTCCTCCAGG SEQ ID NO:360	Identical
miR-340	miR-340GSP	CATGATCAGCTGGGCCAAGAGGCTATAAAG SEQ ID NO:361	miR-340RP	TC+CG+TCTCAGTTAC SEQ ID NO:362	Identical
miR-342	miR-342GSP3	CATGATCAGCTGGGCCAAGAGACGGGTG SEQ ID NO:363	miR-342RP	T+CT+CACACAGAAATCG SEQ ID NO:364	Identical
miR-345	miR-345GSP	CATGATCAGCTGGGCCAAGAGCACTGGACT SEQ ID NO: 484	miR-345RP	T+GC+TGACCCCTAGT SEQ ID NO: 485	one or more base pairs differ
miR-346	miR-346GSP	CATGATCAGCTGGGCCAAGAAGAGGCAGGC SEQ ID NO: 367	miR-346RP	T+GT+CTGCCCGAGTG SEQ ID NO: 488	one or more base pairs differ
miR-363	miR-363 GSP10	CATGATCAGCTGGGCCAAGATACAGATGGA SEQ ID NO:369	miR-363RP	AAT+TG+CAC+GGTATCC SEQ ID NO:370	Identical
miR-370	miR-370GSP	CATGATCAGCTGGGCCAAGACCAGGTTCCA SEQ ID NO:375	miR-370RP	G+CC+TGCTGGGGTGG SEQ ID NO:376	Identical overlapping sequence, ends differ
miR-375	miR-375GSP	CATGATCAGCTGGGCCAAGATCACGCGAGC SEQ ID NO:387	miR-375RP	TT+TG+TTCGTTCGGC SEQ ID NO:388	Identical
miR-376a	miR-376aGSP3	CATGATCAGCTGGGCCAAGAACGTGGAT SEQ ID NO: 467	miR-376aRP2	A+TCGTAGA+GGAAAATCCAC SEQ ID NO: 468	one or more base pairs differ
miR-378	miR-378GSP	CATGATCAGCTGGGCCAAGAACACAGGACC SEQ ID NO:391	miR-378RP	C+TC+CTGACTCCAGG SEQ ID NO:392	Identical

Mouse Target microRNA:	Extension Primer Name	Extension Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Mouse microRNA as compared to Human microRNA
miR-379	miR-379_GSP7	CATGATCAGCTGGGCCAAGATACGTTC SEQ ID NO:393	miR-379RP2	T+GGT+AGACTATGGAACG SEQ ID NO:394	Identical overlapping sequence, ends differ
miR-380-5p	miR-380-5pGSP	CATGATCAGCTGGGCCAAGAGCGCATGTTC SEQ ID NO:395	miR-380-5pRP	T+GGT+TGACCATAGA SEQ ID NO:396	Identical
miR-380-3p	miR-380-3pGSP	CATGATCAGCTGGGCCAAGAAAGATGTGGA SEQ ID NO: 395	miR-380-3pRP	TA+TG+TAGTATGGTCCACA SEQ ID NO: 483	one or more base pairs differ
miR-381	miR-381GSP2	CATGATCAGCTGGGCCAAGAACAGAGAGC SEQ ID NO:399	miR-381RP2	TATA+CAA+GGGCAAGCT SEQ ID NO:400	Identical
miR-382	miR-382GSP	CATGATCAGCTGGGCCAAGACGAATCCACC SEQ ID NO:401	miR-382RP	G+AA+GTTGTTCGTGGT SEQ ID NO:402	Identical
miR-383	miR-383GSP	CATGATCAGCTGGGCCAAGAAGCCACAGTC SEQ ID NO:465	miR-383RP2	A+GATC+AGAAGGTGACTGT SEQ ID NO: 466	one or more base pairs differ
miR-384	miR-384_GSP9	CATGATCAGCTGGGCCAAGATGTGAACAA SEQ ID NO:470	miR-384_RP5	ATT+CCT+AG+AAATTGTTC SEQ ID NO: 471	one or more base pairs differ
miR-410	miR-410 GSP9	CATGATCAGCTGGGCCAAGAACAGGCCAT SEQ ID NO:405	miR-410RP	AA+TA+TAA+CA+CAGATGGC SEQ ID NO:406	Identical
miR-412	miR-412 GSP10	CATGATCAGCTGGGCCAAGAACGGCTAGTG SEQ ID NO:407	miR-412RP	A+CTT+CACCTGGTCCACTA SEQ ID NO:408	Identical
miR-424	miR-424GSP	CATGATCAGCTGGGCCAAGATCCAAAACAT SEQ ID NO:474	miR-424RP2	C+AG+CAGCAATTCATGTTTT SEQ ID NO: 414	one or more base pairs differ
miR-425	miR-425GSP	CATGATCAGCTGGGCCAAGAGGCGGACACG SEQ ID NO:417	miR-425RP	A+TC+GGGAATGTCGT SEQ ID NO:418	Identical
miR-429	miR-429_GSP11	CATGATCAGCTGGGCCAAGAACGGCATTACC SEQ ID NO:479	miR-429RP5	T+AATAC+TG+TCTGGTAATG SEQ ID NO: 480	one or more base pairs differ
miR-431	miR-431 GSP10	CATGATCAGCTGGGCCAAGATGCATGACGG SEQ ID NO:421	miR-431RP	T+GT+CTTGCAGGCCG SEQ ID NO:422	Identical overlapping sequence, ends differ
miR-448	miR-448GSP	CATGATCAGCTGGGCCAAGAATGGGACATC SEQ ID NO:423	miR-448RP	TTG+CATA+TGTAGGATG SEQ ID NO:424	Identical

Mouse Target microRNA:	Extension Primer Name	Extension Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Mouse microRNA as compared to Human microRNA
miR-449	miR-449GSP10	CATGATCAGCTGGGCCAAGAACCAGCTAAC SEQ ID NO:425	miR-449RP2	T+GG+CAGTGTATTGTTAGC SEQ ID NO:426	Identical
miR-450	miR-450GSP	CATGATCAGCTGGGCCAAGATATTAGGAAC SEQ ID NO:427	miR-450RP	TTTT+TG+CGATGTGTT SEQ ID NO:428	Identical
miR-451	miR-451 GSP10	CATGATCAGCTGGGCCAAGAAAACTCAGTA SEQ ID NO:429	miR-451RP	AAA+CCG+TTA+CCATTACTGA SEQ ID NO:430	Identical overlapping sequence, ends differ
let7a	let7a-GSP2	CATGATCAGCTGGGCCAAGAAACTATAC SEQ ID NO:431	let7a-RP	T+GA+GGTAGTAGGTTG SEQ ID NO:432	Identical overlapping sequence, ends differ
let7b	let7b-GSP2	CATGATCAGCTGGGCCAAGAAACCACAC SEQ ID NO:433	let7b-RP	T+GA+GGTAGTAGGTTG SEQ ID NO:432	Identical
let7c	let7c-GSP2	CATGATCAGCTGGGCCAAGAAACCATAC SEQ ID NO:434	let7c-RP	T+GA+GGTAGTAGGTTG SEQ ID NO:432	Identical
let7d	let7d-GSP2	CATGATCAGCTGGGCCAAGAACTATGCA SEQ ID NO:435	let7d-RP	A+GA+GGTAGTAGGTTG SEQ ID NO:436	Identical
let7e	let7e-GSP2	CATGATCAGCTGGGCCAAGAACTATACA SEQ ID NO:437	let7e-RP	T+GA+GGTAGGAGGTTG SEQ ID NO:438	Identical
let7f	let7f-GSP2	CATGATCAGCTGGGCCAAGAAACTATAC SEQ ID NO:439	let7f-RP	T+GA+GGTAGTAGATTG SEQ ID NO:440	Identical overlapping sequence, ends differ
let7g	let7g-GSP2	CATGATCAGCTGGGCCAAGAACTGTACA SEQ ID NO:441	let7g-RP	T+GA+GGTAGTAGTTTG SEQ ID NO:442	Identical
let7i	let7i-GSP2	CATGATCAGCTGGGCCAAGAACAGCACA SEQ ID NO:443	let7i-RP	T+GA+GGTAGTAGTTTG SEQ ID NO:444	Identical

EXAMPLE 5

This Example describes the detection and analysis of expression profiles for three microRNAs in total RNA isolated from twelve different tissues using methods in accordance with an embodiment of the present invention.

Methods: Quantitative analysis of miR-1, miR-124 and miR-150 microRNA templates was determined using 0.5 µg of First Choice total RNA (Ambion, Inc.) per 10 µl primer extension reaction isolated from the following tissues: brain, heart, intestine, kidney, liver, lung, lymph, ovary, skeletal muscle, spleen, thymus and uterus. The primer extension enzyme and quantitative PCR reactions were carried out as described above in EXAMPLE 3, using the following PCR primers:

miR-1 template:

extension primer: CATGATCAGCTGGGCCAAGATACATACTTC (SEQ ID NO: 47)

reverse primer: T+G+GAA+TG+TAAAGAAGT (SEQ ID NO: 48)

forward primer: CATGATCAGCTGGGCCAAGA (SEQ ID NO: 13)

miR-124 template:

extension primer: CATGATCAGCTGGGCCAAGATGGCATTCAC (SEQ ID NO: 149)

reverse primer: T+TA+AGGCACGCGGT (SEQ ID NO: 150)

forward primer: CATGATCAGCTGGGCCAAGA (SEQ ID NO: 13)

miR-150 template:

extension primer: CATGATCAGCTGGGCCAAGACACTGGTA (SEQ ID NO: 213)

reverse primer: T+CT+CCCAACCCTTG (SEQ ID NO: 214)

forward primer: CATGATCAGCTGGGCCAAGA (SEQ ID NO: 13)

Results: The expression profiles for miR-1, miR-124 and miR-150 are shown in FIGURE 3A, 3B, and 3C, respectively. The data in FIGURES 3A-3C are presented in units of microRNA copies per 10 pg of total RNA (y-axis). These units were chosen since human cell lines typically yield ≤ 10 pg of total RNA per cell. Hence the data shown are estimates of microRNA copies per cell. The numbers on the x-axis correspond

to the following tissues: (1) brain, (2) heart, (3) intestine, (4) kidney, (5) liver, (6) lung, (7) lymph, (8) ovary, (9) skeletal muscle, (10) spleen, (11) thymus and (12) uterus.

Consistent with previous reports, very high levels of striated muscle-specific expression were found for miR-1 (as shown in FIGURE 3A), and high levels of brain expression were found for miR-124 (as shown in FIGURE 3B) (see Lagos-Quintana et al., *RNA* P:175-179, 2003). Quantitative analysis reveals that these microRNAs are present at tens to hundreds of thousands of copies per cell. These data are in agreement with quantitative Northern blot estimates of miR-1 and miR-124 levels (see Lim et al., *Nature 433:769-773,* 2005). As shown in FIGURE 3C, miR-150 was found to be highly expressed in the immune-related lymph node, thymus and spleen samples which is also consistent with previous findings (see Baskerville et al., *RNA* 77:241-247, 2005).

While the preferred embodiment of the invention has been illustrated and described, it will be appreciated that various changes can be made therein without departing from the spirit and scope of the invention.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A method for amplifying a microRNA molecule to produce DNA molecules, the method comprising the steps of:

(a) producing a first DNA molecule that is complementary to a target microRNA molecule using primer extension; and

(b) amplifying the first DNA molecule to produce amplified DNA molecules using a universal forward primer and a reverse primer.

2. The method of Claim 1, wherein at least one of the universal forward primer and the reverse primer comprises at least one locked nucleic acid molecule.

3. A method of Claim 1 wherein the primer extension uses an extension primer having a length in the range of from 10 to 100 nucleotides.

4. A method of Claim 1 wherein the primer extension uses an extension primer having a length in the range of from 20 to 35 nucleotides.

5. A method of Claim 1 wherein the extension primer comprises a first portion that hybridizes to a portion of the microRNA molecule.

6. A method of Claim 5 wherein the first portion has a length in the range of from 3 to 25 nucleotides.

7. A method of Claim 5 wherein the extension primer comprises a second portion.

8. A method of Claim 7 wherein the second portion has a length of from 18 to 25 nucleotides.

9. A method of Claim 7 wherein the second portion has a nucleic acid sequence comprising the nucleic acid sequence of SEQ ID NO: 1.

10. A method of Claim 1 wherein the universal forward primer has a length in the range of from 16 nucleotides to 100 nucleotides.

11. A method of Claim 1 wherein the universal forward primer consists of the nucleic acid sequence set forth in SEQ ID NO: 13.

12. A method of Claim 7 wherein the universal forward primer hybridizes to the complement of the second portion of the extension primer.

13. A method of Claim 2 wherein the universal forward primer comprises at least one locked nucleic acid molecule.

14. A method of Claim 13 wherein the universal forward primer comprises from 1 to 25 locked nucleic acid molecules.

15. A method of Claim 1 wherein the reverse primer has a length in the range of from 10 nucleotides to 100 nucleotides.

16. A method of Claim 2 wherein the reverse primer comprises at least one locked nucleic acid molecule.

17. A method of Claim 16 wherein the reverse primer comprises from 1 to 25 locked nucleic acid molecules.

18. A method of Claim 1 wherein the reverse primer is selected to specifically hybridize to a DNA molecule complementary to a selected microRNA molecule under defined hybridization conditions.

19. A method of Claim 1 further comprising the step of measuring the amount of amplified DNA molecules.

20. A method of Claim 1 wherein amplification is achieved by multiple successive PCR reactions.

21. A method for measuring the amount of a target microRNA in a sample from a living organism, the method comprising the step of measuring the amount of a target microRNA molecule in a multiplicity of different cell types within a living organism, wherein the amount of the target microRNA molecule is measured by a method comprising the steps of:

(1) producing a first DNA molecule complementary to the target microRNA molecule in the sample using primer extension;

(2) amplifying the first DNA molecule to produce amplified DNA molecules using a universal forward and a reverse primer; and

(3) measuring the amount of the amplified DNA molecules.

22. The method of Claim 21, wherein at least one of the universal forward primer and the reverse primer comprises at least one locked nucleic acid molecule.

23. The method of Claim 21, wherein the amount of the amplified DNA molecules are measured using fluorescence-based quantitative PCR.

24. The method of Claim 21, wherein the amount of the amplified DNA molecules are measured using SYBR green dye.

25. A kit for detecting at least one mammalian target microRNA comprising at least one primer set specific for the detection of a target microRNA, the primer set comprising:

(1) an extension primer for producing a cDNA molecule complementary to a target microRNA, the extension primer comprising a first portion that hybridizes to a target microRNA and a second portion having a hybridization sequence for a universal forward PCR primer;

(2) a universal forward PCR primer for amplifying the cDNA molecule, comprising a sequence selected to hybridize to the hybridization sequence on the extension primer; and

(3) a reverse PCR primer for amplifying the cDNA molecule, comprising a sequence selected to hybridize to a portion of the cDNA molecule.

26. The kit according to Claim 25, wherein at least one of the universal forward and reverse PCR primers includes at least one locked nucleic acid molecule.

27. The kit according to Claim 25, wherein the extension primer has a length in the range of from 10 to 100 nucleotides.

28. The kit according to Claim 25, wherein the first portion of the extension primer has a length in the range of from 3 to 25 nucleotides.

29. The kit according to Claim 25, wherein the second portion of the extension primer has a length in the range of from 18 to 25 nucleotides.

30. The kit according to Claim 25, wherein the second portion of the extension primer has a nucleic acid sequence comprising the nucleic acid sequence of SEQ ID NO: 1.

31. The kit according to Claim 25, wherein the universal forward PCR primer has a length in the range of from 16 to 100 nucleotides.

32. The kit according to Claim 25, wherein the universal forward primer consists of the nucleic acid sequence set forth in SEQ ID NO: 13.

33. The kit according to Claim 25, wherein the reverse PCR primer has a length in the range of from 10 to 100 nucleotides.

34. The kit according to Claim 25, wherein the reverse PCR primer comprises from 1 to 25 locked nucleic acid molecules.

35. The kit according to Claim 25, wherein the at least one mammalian target microRNA is a human microRNA.

36. The kit according to Claim 35, wherein the at least one target microRNA is selected from the group consisting of miR-1, miR-7, miR-9*, miR-10a, miR-10b, miR-15a, miR-15b, miR-16, miR-17-3p, miR-17-5p, miR-18, miR-19a, miR-19b, miR-20, miR-21, miR-22, miR-23a, miR-23b, miR-24, miR-25, miR-26a, miR-26b, miR-27a, miR-28, miR-29a, miR-29b, miR-29c, miR-30a-5p, miR-30b, miR-30c, miR-30d, miR-30e-5p, miR-30e-3p, miR-31, miR-32, miR-33, miR-34a, miR-34b, miR-34c, miR-92, miR-93, miR-95, miR-96, miR-98, miR-99a, miR-99b, miR-100, miR-101, miR-103, miR-105, miR-106a, miR-107, miR-122, miR-122a, miR-124, miR-124, miR-124a, miR-125a, miR-125b, miR-126, miR-126*, miR-127, miR-128a, miR-128b, miR-129, miR-130a, miR-130b, miR-132, miR-133a, miR-133b, miR-134, miR-135a, miR-135b, miR-136, miR-137, miR-138, miR-139, miR-140, miR-141, miR-142-3p, miR-143, miR-144, miR-145, miR-146, miR-147, miR-148a, miR-148b, miR-149, miR-150, miR-151, miR-152, miR-153, miR-154*, miR-154, miR-155, miR-181a, miR-181b, miR-181c, miR-182*, miR-182, miR-183, miR-184, miR-185, miR-186, miR-187, miR-188, miR-189,

miR-190, miR-191, miR-192, miR-193, miR-194, miR-195, miR-196a, miR-196b, miR-197, miR-198, miR-199a*, miR-199a, miR-199b, miR-200a, miR-200b, miR-200c, miR-202, miR-203, miR-204, miR-205, miR-206, miR-208, miR-210, miR-211, miR-212, miR-213, miR-213, miR-214, miR-215, miR-216, miR-217, miR-218, miR-220, miR-221, miR-222, miR-223, miR-224, miR-296, miR-299, miR-301, miR-302a*, miR-302a, miR-302b*, miR-302b, miR-302d, miR-302c*, miR-302c, miR-320, miR-323, miR-324-3p, miR-324-5ρ, miR-325, miR-326, miR-328, miR-330, miR-331, miR-337, miR-338, miR-339, miR-340, miR-342, miR-345, miR-346, miR-363, miR-367, miR-368, miR-370, miR-371, miR-372, miR-373*, miR-373, miR-374, miR-375, miR-376b, miR-378, miR-379, miR-380-5p, miR-380-3p, miR-381, miR-382, miR-383, miR-410, miR-412, miR-422a, miR-422b, miR-423, miR-424, miR-425, miR-429, miR-431, miR-448, miR-449, miR-450, miR-451, Iet7a, Iet7b, Iet7c$_5$ Iet7d, Iet7e, Iet7f, Iet7g, Iet7i, miR-376a, and miR-377.

37. The kit according to Claim 35, wherein the at least one target microRNA is selected from the group consisting of: miR-1, miR-7, miR-10b, miR-26a, miR-26b, miR-29a, miR-30e-3p, miR-95, miR-107, miR-141, miR-143, miR-154*, miR-154, miR-155, miR-181a, miR-181b, miR-181c, miR-190, miR-193, miR-194, miR-195, miR-202, miR-206, miR-208, miR-212, miR-221, miR-222, miR-224, miR-296, miR-299, miR-302c*, miR-302c, miR-320, miR-339, miR-363, miR-376b, miR-379, miR-410, miR-412, miR-424, miR-429, miR-431, miR-449, miR-451, Iet7a, Iet7b, Iet7c, Iet7d, Iet7e, Iet7f, Iet7g, and Iet7i.

38. The kit according to Claim 25, wherein the at least one target microRNA is a murine microRNA.

39. A kit for detecting at least one mammalian microRNA comprising at least one oligonucleotide primer selected from the group consisting of SEQ ID NO: 2 to SEQ ID NO:499.

40. The kit according to Claim 39 comprising at least one or more oligonucleotide primers selected from the group consisting of SEQ ID NOS: 47, 48, 49, 50, 55, 56, 81, 82, 83, 84, 91, 92, 103, 104, 123, 124, 145, 146, 193, 194, 197, 198, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 239, 240, 247, 248, 253, 254, 255, 256, 257,

258, 277, 278, 285, 286, 287, 288, 293, 294, 301, 302, 309, 310, 311, 312, 315, 316, 317, 318, 319, 320, 333, 334, 335, 336, 337, 338, 359, 360, 369, 370, 389, 390, 393, 394, 405, 406, 407, 408, 415, 416, 419, 420, 421, 422, 425, 426, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 461 and 462.

41. An oligonucleotide primer for detecting a human microRNA selected from the group consisting of SEQ ID NO: 2 to SEQ ID NO: 499.

42. An oligonucleotide primer according to Claim 41, wherein the primer is selected from the group consisting of SEQ ID NO: 47, 48, 49, 50, 55, 56, 81, 82, 83, 84, 91, 92, 103, 104, 123, 124, 145, 146, 193, 194, 197, 198, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 239, 240, 247, 248, 253, 254, 255, 256, 257, 258, 277, 278, 285, 286, 287, 288, 293, 294, 301, 302, 309, 310, 311, 312, 315, 316, 317, 318, 319, 320, 333, 334, 335, 336, 337, 338, 359, 360, 369, 370, 389, 390, 393, 394, 405, 406, 407, 408, 415, 416, 419, 420, 421, 422, 425, 426, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 461 and 462.

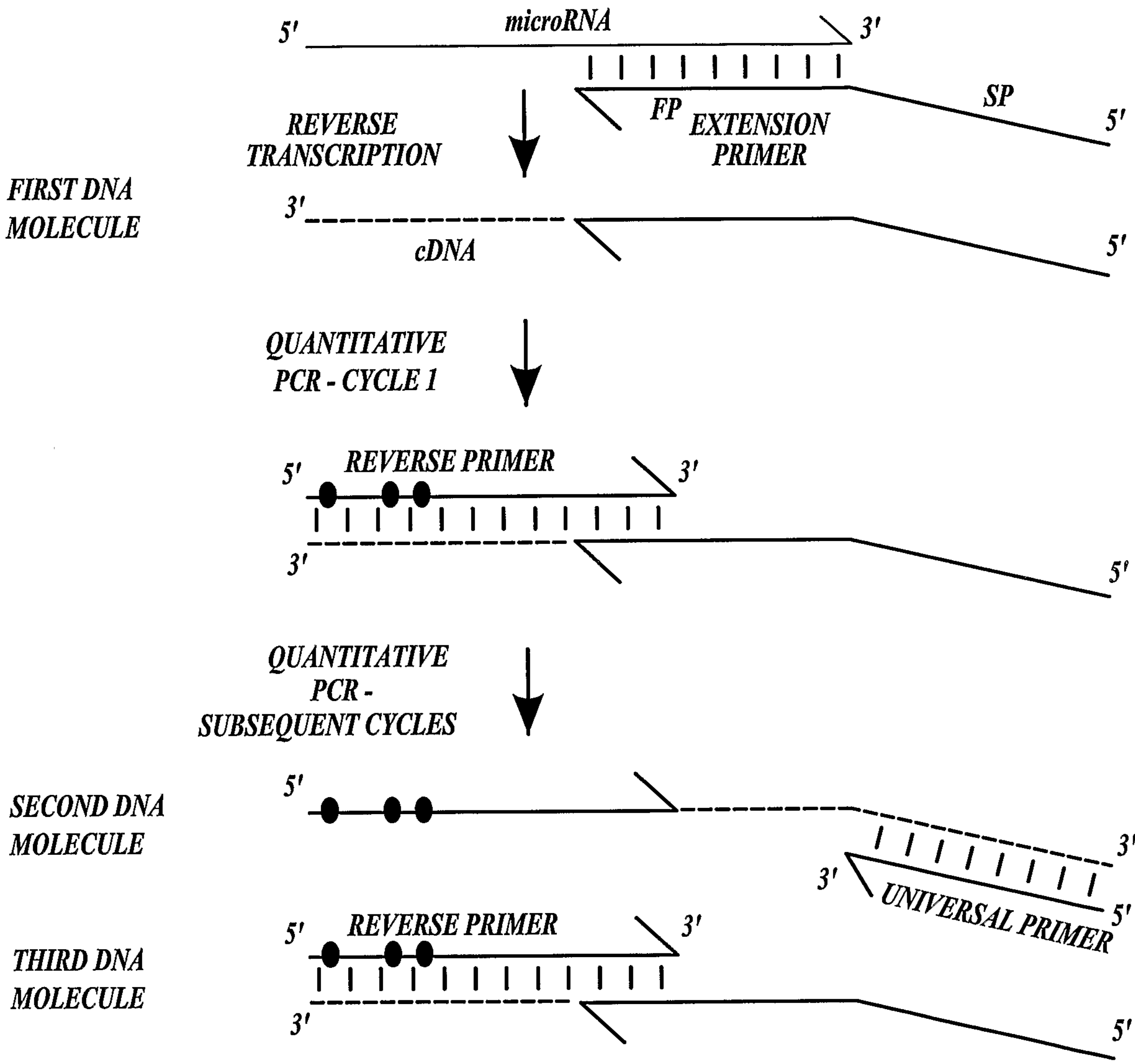
5'
microRNA
3'
SP
FP
EXTENSION PRIMER
5'
REVERSE TRANSCRIPTION
FIRST DNA MOLECULE
3'
cDNA
5'
QUANTITATIVE PCR - CYCLE 1
REVERSE PRIMER
5'
3'
3'
5'
QUANTITATIVE PCR - SUBSEQUENT CYCLES
SECOND DNA MOLECULE
5'
3'
3'
UNIVERSAL PRIMER
5'
THIRD DNA MOLECULE
REVERSE PRIMER
5'
3'
3'
5'

Fig.1.

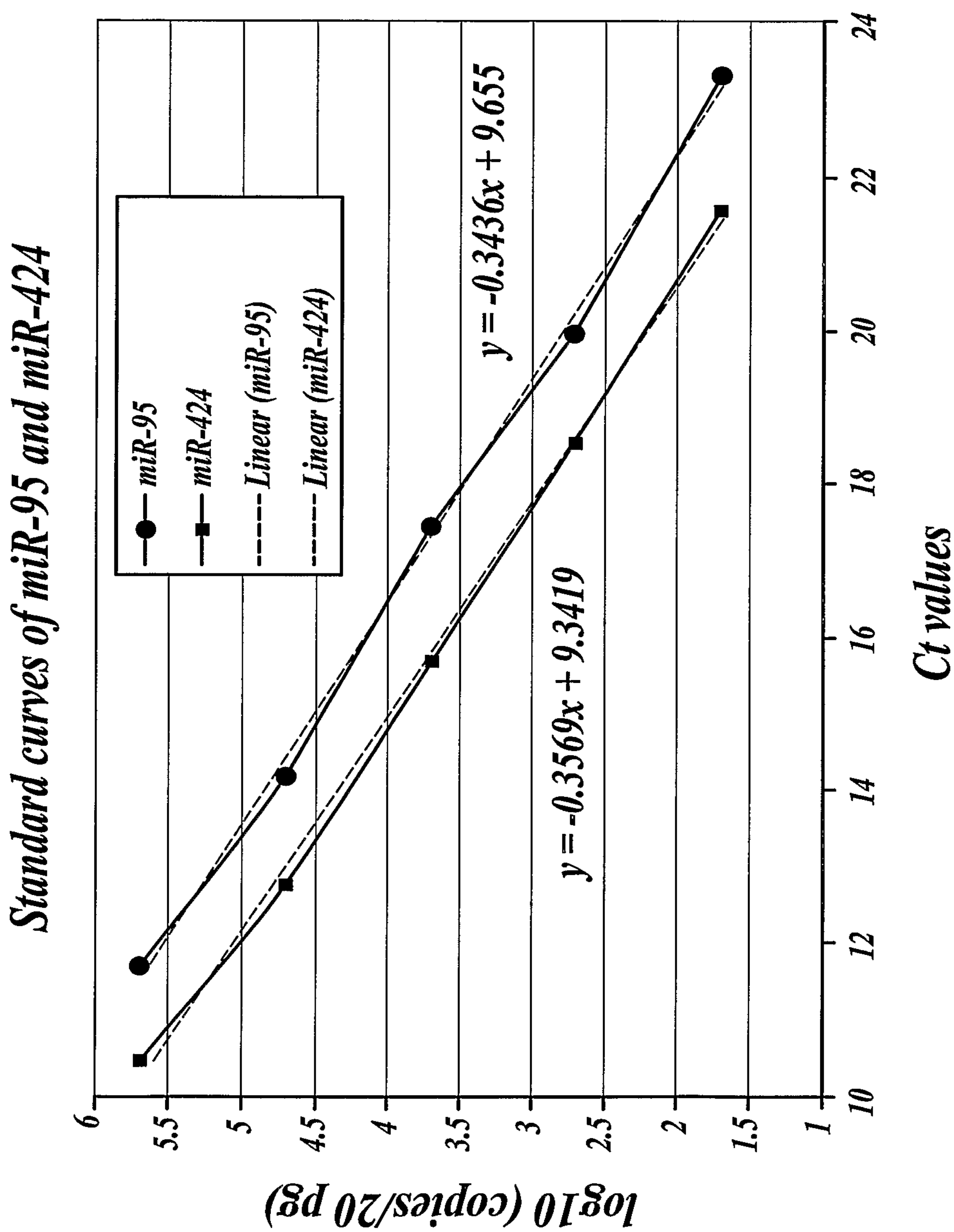
Standard curves of miR-95 and miR-424
miR-95
miR-424
Linear (miR-95)
Linear (miR-424)
y = -0.3436x + 9.655
y = -0.3569x + 9.3419
log10 (copies/20 pg)
Ct values
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
10
12
14
16
18
20
22
24

Fig.2.

Fig.3A.

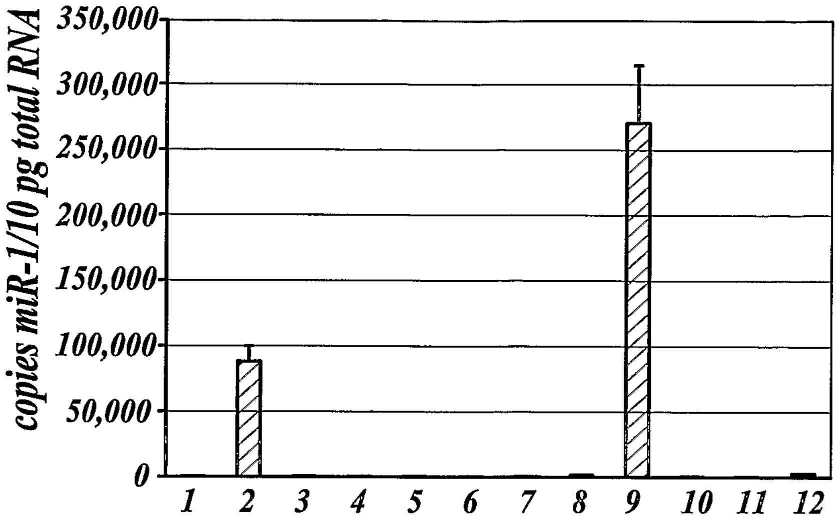

Fig.3B.

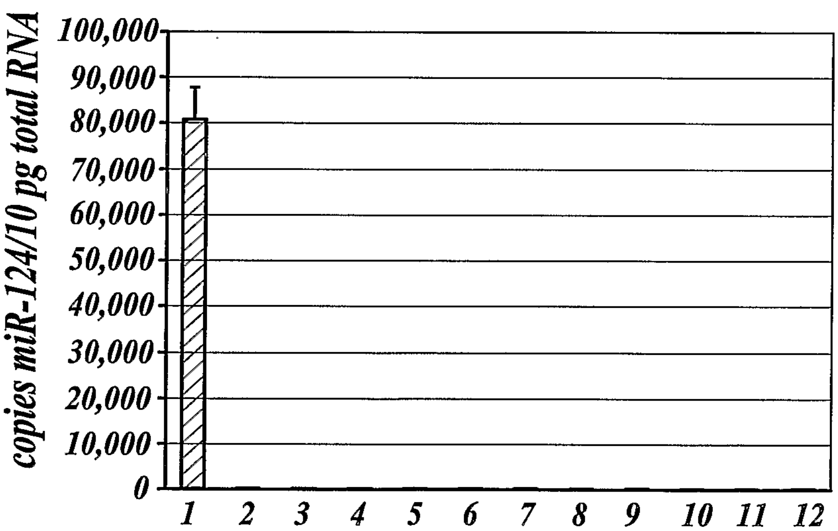

Fig.3C.